THE PROTEINS OF THE WHEAT KERNEL

BY

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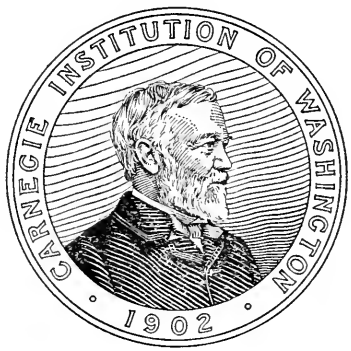

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THE PROTEINS OF THE WHEAT KERNEL.

INTRODUCTION.

Of the protein substances used as food none is of more importance than those contained in the seeds of wheat. Although these bodies attracted the attention of investigators more than one hundred years ago and have since then been many times the subject of study, the published statements respecting them are so conflicting and uncertain that it has heretofore been impossible to know what the truth regarding them actually is. With the purpose of clearing up the existing confusion and determining the real value of the evidence offered, as well as extending, as far as possible, our knowledge of these important substances, the writer some years ago undertook an investigation of this seed which has recently been concluded by work done under grants from the Carnegie Institution of Washington. As the results of these investigations have been published from time to time in a number of different papers, appearing in four different journals, it has been thought desirable to bring all this work together in one paper. In so doing the details have been reproduced in full, for the nature of the evidence is such that its value largely consists in concordant results of many experiments, repeated under different conditions, since it is not yet possible to establish the chemical individuality of different protein substances by demonstrating their possession of definite physical properties, as may be done with the simpler organic compounds.

The experience of the writer in his endeavors to understand and repeat the work of many of his predecessors has made him feel the importance of these details to future workers along the same lines and is his excuse for giving with so much minuteness the results of his own work, which to those not familiar with the difficulties of the subject must appear to a large extent unnecessary. In order to make this work available to those who wish simply to know the results, a comprehensive summary of this paper is given at the end of this publication, and the details of the many operations and experiments need be read only by those who wish to become familiar with these.

The account of this work is preceded by a review of the literature of the subject, from which an idea of the unsatisfactory state of our previous

knowledge can be obtained. This review is interesting, also, as it shows the slow development of the study of vegetable proteins and how the several investigators have been influenced by the knowledge of the animal proteins prevailing at the time the work was done. In carrying out his investigations of these proteins the writer has received the assistance of Messrs. Voorhees, Campbell, Harris, and Clapp, for which he wishes here to make acknowledgment; but especially is he indebted to Prof. S. W. Johnson, under whose direction and with whose advice and encouragement this work was first undertaken in the laboratory of the Connecticut Agricultural Experiment Station, where it has since been continued.

REVIEW OF THE LITERATURE.

The fact that gluten can be obtained from wheat flour by washing with water appears to have been first published by Beccari.[1]

That alcohol extracts a protein substance from wheat flour was first stated by Einhof,[2] who considered this to be the same as the gluten.

Taddei[3] found that gluten consists of two substances, one of which is soluble in alcohol, which he named "gliadin," the other insoluble in alcohol, which he named "zymom."

De Saussure[4] obtained from wheat gluten about 72 per cent of plant-albumin in the insoluble form, about 20 per cent of plant-gelatin, or, as he proposed to call it, "glutin," and about 1 per cent of mucin, which latter substance he considered to be similar to the mucin described by Berzelius.

Berzelius[5] thought that the alcoholic extract contained another protein substance, which he called "mucin," and that the part of the gluten which was insoluble in alcohol was so similar to albumin that he called this "plant-albumin."

Boussingault[6] agreed with Einhof that the part of the gluten that was soluble in alcohol was the same as the entire gluten protein.

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[1] Beccari. Reference to this publication has, for many years, appeared in the literature as Comon. Bonon. I. 1, p. 122. It should be De Bononiensi Scientiarum et Artium Instituto atque Academia Commentarii, 1745, II, part 1, p. 122. In this paper Beccari refers to the fact that in 1728 he had orally communicated to the Academy the previously unpublished fact that wheat flour can be separated into two parts, one of vegetable, the other of animal character. The substance of this communication was published in the above-cited paper in which the separation of gluten from wheat flour, by washing with water, is described.

[2] Einhof, Journal der Chemie von Gehlen, 1805, V, p. 131.

[3] Taddei, Annals of Philosophy, 1820, XV, p. 390.

[4] De Saussure, Schweiger's Journal, 1833, LXIX, p. 188.

[5] Berzelius, Lehrbuch der Chimie, Auflage 3, 1837, VI, p. 453.

[6] Boussingault, Annales de Chimie et de Physique, 1838, LXV, p. 30.

Liebig[1] named the part of the gluten that was insoluble in alcohol "plant-fibrin," on account of its supposed resemblance to blood-fibrin. The substance soluble in alcohol he called "plant-gelatin" and considered it to be a compound of a protein with an organic acid. In the aqueous extract of the flour he recognized the presence of albumin.

Scherer[2] prepared plant-fibrin by dissolving gluten in dilute alkali, filtering, neutralizing with acetic acid, and extracting the precipitate with hot alcohol and then with ether.

Bonchardat[3] considered that wheat gluten contained a protein soluble in extremely dilute acids, which he named "albuminose."

Dumas & Cahours[4] found four protein substances in wheat flour, namely, plant-fibrin, which remained after extracting gluten with alcohol; a substance which they considered similar to casein, which was deposited by cooling the alcoholic extract; glutin, which was obtained by concentrating and cooling the alcoholic extract, and albumin, which was present in the aqueous washings of the gluten and was coagulated by boiling. The plant-fibrin they considered to be identical with blood-fibrin, as both had the same ultimate composition, and the albumin to be identical with egg-albumin for the same reason.

Mulder[5] considered the plant-gelatin, obtained by extracting gluten with alcohol, to be a compound of sulphur with "protein," which contained the same proportion of sulphur as blood-albumin.

Von Bibra[6] recognized three proteins in gluten—plant-fibrin, which formed 70.8 per cent; plant-gelatin, 16.2 per cent; and plant-casein, 7.1 per cent. In the water used for washing out the gluten he found 1.34 per cent of albumin.

Günsberg[7] considered that Taddei's view that there were only two proteins in wheat gluten was correct. By boiling wheat gluten with water he obtained five preparations which separated on cooling and had the same ultimate composition as has been established for gliadin. By treating gliadin in the same way he obtained a body of the same composition. The substance which Günsberg thus obtained was unquestionably gliadin, which is sparingly soluble in hot water, and he appears to have been the first to obtain correct analyses of this protein.

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[1] Liebig, Annalen der Chemie und Pharmacie, 1841, XXXIX, p. 129.
[2] Scherer, Annalen der Chemie und Pharmacie, 1841, XL, p. 7.
[3] Bonchardat, ibid., 1842, XLII, p. 124.
[4] Dumas & Cahours, Journal für praktische Chemie, 1843, XXVIII, p. 398.
[5] Mulder, Annalen der Chemie und Pharmacie, 1844, LII, p. 419.
[6] Von Bibra, Die Getreidearten und das Brod, Nüremberg, 1860.
[7] Günsberg, Journal für praktische Chemie, 1862, LXXXV, p. 213.

Commaille[1] recognized, as protein constituents of flour, sitosin, soluble in water and coagulable by heat; imesin, soluble, after drying, only in water containing 0.1 per cent hydrochloric acid; sitesin, soluble in 0.1 per cent hydrochloric acid; glutin, nearly insoluble in dilute acid, easily in strong acid, forming an emulsion with alcohol, which is separated by much water; and mucin, which dissolves easily, even after drying, in water and in cold 80 per cent alcohol.

Ritthausen[2] next published the results of his extensive investigations in a volume in which he discussed at length the proteins of the wheat kernel. The composition, properties, and the proportions in which they occur in the seed were given in detail, and also the evidence which he considered showed the individuality of each. He recognized five proteins, namely, gluten-casein, gluten-fibrin, plant-gelatin or gliadin, and mucedin, as constituents of the gluten, and also albumin, which he found in the aqueous extracts of the seed.

The gluten-casein was not soluble in water, very slightly soluble in dilute alcohol, and readily soluble in very dilute acids and alkalis. When decomposed by boiling with sulphuric acid it yielded tyrosine, leucine, 5.3 per cent of glutaminic acid, and 0.33 per cent of aspartic acid. In the dry gluten he found from 26 to 31.4 per cent of gluten-casein, which he considered to be minimal quantities.

Table giving results as ascertained by Ritthausen.

	Gluten-casein.	Gluten-fibrin.	Plant-gelatin.	Mucedin.	Albumin.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.94	54.31	52.76	54.11	53.12
Hydrogen	7.04	7.18	7.10	6.90	7.18
Nitrogen	17.14	16.89	18.01	16.63	17.60
Sulphur	0.96	1.01	0.85	0.88	1.55
Oxygen	21.92	20.61	21.37	21.48	20.55
	100.00	100.00	100.00	100.00	100.00

The gluten-fibrin formed that fraction of the alcohol-soluble proteins which was soluble in the strongest alcohol and separated from a hot concentrated solution in 50 to 60 per cent alcohol on cooling. Owing to the difficulty encountered in separating gluten-fibrin from the other alcohol-soluble proteins, it was impossible to determine its amount. Usually from

[1] Commaille, Journal de Pharmacie, 1866 (4), IV, p. 108.
[2] Ritthausen, Die Eiweisskörper, etc., Bonn, 1872.

2 to 3 per cent of the gluten was obtained, which corresponds to 0.25 to 0.35 per cent of the flour. The actual quantity he considered to be much more, and that in different varieties of wheat the proportion of gluten-fibrin varied greatly.

Ritthausen described gluten-fibrin as insoluble in water, but by boiling with water it was decomposed and rendered insoluble in alcohol. In alcohol of 30 to 70 per cent it dissolved readily when heated, and separated again on cooling, more completely from the more dilute alcohol. From dilute solutions on concentration and from concentrated solutions on cooling the gluten-fibrin separated on the surface of the liquid as a thick, soft skin, which was renewed as often as it was removed, which property Ritthausen considered distinguished it from mucedin and plant-gelatin. In cold alcohol of 80 to 90 per cent the gluten-fibrin was soluble to a considerable degree. Dilute acids and alkalis dissolved this protein freely, yielding solutions from which it was precipitated on neutralizing to a slight acid reaction.

The composition of gluten-fibrin is shown in the table on page 8.

Plant-gelatin or gliadin formed the fraction of the alcohol-soluble protein that dissolved freely in alcohol of 60 to 70 per cent. The solubility of this protein decreased rapidly when the proportion of alcohol to water fell below or above this strength. It was very slightly soluble in cold water, more so in hot water. By boiling with water it was gradually rendered insoluble in alcohol. Extremely dilute acids and alkalis dissolved plant-gelatin readily.

The amount of plant-gelatin which different wheats contain was not determined, owing to the impossibility of separating it from the other proteins. The composition of this protein is shown in the preceding table.

Mucedin formed the fraction of the alcohol-soluble proteins which was soluble in the most dilute alcohol. Except for its greater solubility in water and in very dilute alcohol, mucedin does not appear to differ greatly in its properties from plant-gelatin. Its composition is given in the table on page 8.

Mucedin yielded 25 per cent of glutaminic acid when boiled with sulphuric acid, but other decomposition products were not determined. Only a very small quantity of mucedin was obtained in a pure state, and no estimate of its total amount was made. The relative proportion, however, Ritthausen considered to vary greatly in different sorts of wheat.

Albumin was obtained by heating the acidified wash-waters of the gluten. This, however, he considered as possibly derived from the soluble part of the gluten-proteins. The composition of this albumin is given in the table on page 8.

Weyl[1] was the first to recognize the presence of globulin in wheat flour, and states that besides vegetable-vitellin, vegetable-myosin, which coagulated at 55° to 60°, was also present.

Weyl & Bischoff[2] considered the protein matter of wheat to consist chiefly of a myosin-like globulin which they called vegetable-myosin, and that, if so, this must be the substance from which gluten is derived, for other proteins are present only in small quantity. Extraction with 15 per cent salt solution left a residue from which they obtained no gluten. They therefore considered it probable that the gluten forms from the myosin in consequence of a ferment action similarly to the formation of fibrin from fibrinogen. No ferment, however, could be detected. They also found that large amounts of sodium chloride, sodium sulphate, and magnesium sulphate hindered the formation of gluten in the same way that sodium and magnesium sulphates hinder the formation of fibrin. As no gluten was obtained from flour extracted with alcohol, they concluded that the myosin had been coagulated. By warming flour 48 to 96 hours below 60°, the coagulation point of myosin, no gluten was obtained from the meal after adding a little unwarmed flour, showing that the gluten-forming substance had been coagulated.

Balland[3] found that nearly the same amount of gluten was formed with water at 2°, 15°, and 60°, and therefore concluded that no ferment action took part in its formation.

According to Martin,[4] alcohol extracts from gluten but one protein substance. This is soluble in hot water, but insoluble in cold ; hence is insoluble phytalbumose. The residue remaining after treatment with alcohol is uncoagulated protein, soluble in dilute acids and alkalis. This he called "gluten-fibrin." The insoluble phytalbumose is not present, as such, in flour, since direct extraction of the meal with 75 per cent alcohol removes no protein. Extraction with water yields less globulin and soluble albumose than extraction with sodium chloride solution of 10 to 15 per cent. Martin therefore concluded that the insoluble phytalbumose is formed from the soluble by the action of water, the gluten-fibrin being formed by a similar action of water on the globulin ; that is, conversion into an albuminate. This albuminate and the insoluble phytalbumose together constitute gluten.

W. Johannsen[5] believed that there was no ferment action in the formation of gluten. Dough was obtained by grinding dried gluten and mixing with starch, and also by mixing moist gluten with starch.

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[1] Weyl, Zeitschrift für physiologische Chemie, 1877, I, p. 72.
[2] Weyl & Bischoff, Berichte der deutschen chemischen Gesellschaft, 1880, XIII, p. 367.
[3] Balland, Comptes rendus de l'Académie des Sciences, 1883, CXV, p. 202.
[4] Martin, British Medical Journal, 1886, II, p. 104.
[5] W. Johannsen, Annales Agronomiques, 1888, XIV, p. 420.

Chittenden & Smith[1] prepared many samples of gluten-casein according to Ritthausen's method. As an average of eight analyses they found the following composition:

	P. ct.
Carbon	52.87
Hydrogen	6.99
Nitrogen	15.86
Sulphur	1.17
Oxygen	23.11
	100.00

They also prepared and analyzed the various products of peptic digestion of this protein.

Osborne & Voorhees[2] investigated the number and character of the proteins of wheat, but as their results are given in detail in the body of this paper they need not here be further mentioned.

O'Brien[3] recognized globulin, proteose, and the gluten-proteins in extracts of wheat flour. The protein-leucosin, which Osborne & Voorhees considered to be an albumin, was regarded as a globulin by O'Brien, since it is precipitated by saturating its solutions with magnesium sulphate.

About 1 per cent of the flour consists of proteins soluble in saline solutions and coagulating on boiling. Neither ferment action nor globulin take part in gluten formation. Gluten consists of zymom, insoluble in alcohol, and glian, soluble therein. Glian is formed by hydration of the protein of the flour and zymom from glian by further hydration. Glian yields myxon, glutine, and mucine, which are not constituents of glian, but derived from it.

Frankfurt[4] estimated the proportion of various constituents of the embryo of wheat, and found globulin 21.62 per cent and albumose 13.62 per cent.

O'Brien[5] stated that the proteins of the wheat embryo consist of globulins of the myosin type, coagulating at 55°, soluble in dilute solutions of sodium chloride or magnesium sulphate and precipitated by excess of these salts; globulins of the vitellin type, coagulating at 75° to 78°, and soluble in dilute solution of sodium chloride, but not precipitated by an excess; proteose and albumin, not coagulating below 80°, soluble in sodium chloride solution, but not precipitated by an excess, nor by dialysis, nor by carbonic acid.

Kjeldahl[6] found that all of a number of preparations of the alcohol-soluble protein made from wheat flour showed an almost constant content of about 52 per cent of carbon and 17.25 per cent of nitrogen, and when dissolved in

[1] Chittenden & Smith, Journal of Physiology, 1890, XI, p. 419.
[2] Osborne & Voorhees, American Chemical Journal, 1893, XV, p. 392.
[3] O'Brien, Annals of Botany, 1895, IX, p. 171.
[4] Frankfurt, Versuchs-Stationen, 1895, XLVII, p. 449.
[5] O'Brien, Annals of Botany, 1895, IX, p. 543.
[6] Kjeldahl, Agricultur chemischen Centralblatt, 1896, XXV, p. 197.

75 per cent alcohol a specific rotation of —92°. This rotation was so constant, not only for the protein from different sorts of wheat from different regions, but also for those from crops of four different years, that it seemed to Kjeldahl that wheat flour contained only one single protein substance soluble in alcohol.

Fleurent[1] held the view that only one protein substance soluble in alcohol was present in wheat flour, and proposed a method for determining the amount of gliadin and glutenin. By this method he found that gluten contained from 60 to 80 per cent of gliadin and 18 to 25 per cent of glutenin, according to the variety of the wheat from which it was obtained.

Guthrie[2] concluded that the water-absorbing power of wheat flour was greater when the proportion of glutenin to gliadin was greater, strong flours being those relatively rich in glutenin.

Teller[3] devised methods for determining the relative quantities of the different proteins in wheat flour and applied them to flours of different origin and to various mill products.

Teller[4] also concluded that the proteose found by Osborne & Voorhees was gliadin that had been dissolved in small quantity in the aqueous extract. Osborne[5] showed that this was erroneous and gave the reasons why he had not mistaken gliadin for proteose.

Morishima's[6] investigations led him to believe that wheat gluten contained but a single protein, and that glutenin and gliadin were derivatives of one and the same substance, which he named artolin.

Teller[7] determined the proportion of the several proteins present in the wheat kernel on many consecutive days during the ripening of the grain and found a large increase of gliadin nitrogen during this period, together with a smaller though marked decrease of the glutenin nitrogen when considered in proportion to the whole amount of nitrogen present. The changes in the proportion of leucosin and globulin nitrogen were less marked and more irregular.

Ritthausen[8] again asserted his belief in the existence of three distinct proteins in wheat that were soluble in alcohol, but offered no new evidence of their existence.

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[1] Fleurent, Comptes rendus de l'Académie des Sciences, 1896, CXXIII, p. 755.
[2] Guthrie, Agricultural Gazette of New South Wales, September, 1896.
[3] Teller, Arkansas Agr. Exp. Sta. Bull. 42, part 2, p. 81. 1896.
[4] Teller, American Chemical Journal, 1897, XIX, p. 65.
[5] Osborne, ibid., 1897, XIX, p. 263.
[6] Morishima, Archiv für experim. Pathologie und Pharmakologie, 1898, XLI, p. 348.
[7] Teller, Arkansas Agr. Exp. Sta., Bull. 53, p. 53. 1898.
[8] Ritthausen, Journal für praktische Chemie, 1899, LIX, p. 474.

Snyder[1] determined the amount of the different proteins in various flours and mill products. He found 73.9 per cent of the total protein to be gliadin in patent flour from soft winter wheat and 63.7 per cent in that from hard winter wheat. He concluded that the protein in the gluten of a flour good for bread-making consists of 65 per cent of gliadin and 35 per cent of glutenin. The ratio of gliadin to glutenin in different grades of flour varies between 1 to 4 and nearly 2 to 1. While the lower grades of flour contain more protein than the higher, the proportion of gliadin to glutenin is not such as to produce bread of the best physical properties.

Osborne & Campbell[2] found that the leucosin, globulin, and proteose, obtained in very small quantity from the entire wheat kernel, together constitute nearly the whole of the protein of the embryo, and that gliadin and glutenin, which are the principal proteins of the endosperm, could not be obtained from the embryo. The details of this investigation are given in full in subsequent pages of this paper.

Kossel & Kutscher,[3] following Ritthausen's directions, prepared the proteins of wheat gluten and determined the proportion of basic products which they yielded on decomposition with acids. They found that glutenin was sharply distinguished from the protein soluble in alcohol by the fact that it yields a notable quantity of lysine, whereas all their products derived from the alcoholic extract of gluten yielded none of this diamino-acid. They held the view, advanced by Ritthausen, that in gluten there are three protein substances soluble in alcohol. Of these mucedin yielded 3.13, gliadin 2.75, and gluten-fibrin 3.05 per cent of arginine and 0.43, 1.20, and 1.53 per cent respectively of histidine; but in view of the methods employed for the determinations of these bases they consider these differences too small to justify the conclusion that these are distinct protein substances.

Dennstedt[4] decomposed "wheat fibrin" by boiling with baryta and found that one-third of the nitrogen was split off as ammonia and one-fifth of the sulphur as sulphide and sulphate. After removing the barium and treating the solution with lead acetate he separated proteoses, which he analyzed.

Osborne[5] made careful determinations of total sulphur in four samples of thoroughly purified gliadin and found an average of 1.027 per cent, of which 0.619 per cent was split off as sulphide by boiling with caustic alkali.

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[1] Snyder, Minnesota Agr. Exp. Sta., Bull. 63. 1899.
[2] Osborne & Campbell, Journal American Chemical Society, 1899, XXI, p. 486.
[3] Kossel & Kutscher, Zeitschrift für physiologische Chemie, 1901, XXXI, p. 165.
[4] Dennstedt, Chemiker Zeitung, 1901, p. 5.
[5] Osborne, Journal American Chemical Society, 1902, XXIV, p. 140.

Osborne & Harris,[1] in a study of the different forms of binding of nitrogen in proteins, found that leucosin of wheat yielded 1.16 per cent of nitrogen as ammonia and 3.50 per cent of nitrogen in basic compounds, precipitable by phosphotungstic acid; that the globulin yielded 1.42 per cent of nitrogen as ammonia and 6.83 per cent of basic nitrogen; that gliadin yielded 4.3 per cent of nitrogen as ammonia and 1.09 per cent of basic nitrogen, and that glutenin yielded 3.31 per cent of nitrogen as ammonia and 2.05 per cent of basic nitrogen. They also found[2] that, while none of the wheat proteins yielded any furfurol on distillation with hydrochloric acid, glutenin gave a moderately strong, gliadin a strong, and leucosin a very strong reaction with the Molisch test. This test is commonly regarded as giving evidence of a carbohydrate complex in the protein molecule, but they decided that other evidence is necessary before such a conclusion is justified.

Osborne & Harris[3] found the specific rotation of gliadin dissolved in alcohol of 80 per cent by volume to be $-91.9°$ and $-92.5°$. In comparing the tryptophane reaction of many proteins these same authors found[4] that the globulin of wheat gave only a slight reaction, gliadin and glutenin one of medium intensity, and leucosin the strongest reaction of all the proteins examined, thus indicating the relative amounts of tryptophane or indol-amino-propionic acid which these proteins yield on decomposition with acid.

Kutscher[5] determined the amount of tyrosine and glutaminic acid yielded by the proteins of wheat gluten when decomposed by boiling with sulphuric acid. In glutenin he found 2.75, in gluten-fibrin 4.43, in gliadin 2.09, and in mucedin 2.35 per cent of tyrosine; in glutenin 9, in gluten-fibrin 13.07, in gliadin 18.54, and in mucedin 19.81 per cent of glutaminic acid.

Naysmith[6] found that gluten contained 0.12 per cent of phosphorus. Gliadin extracted by 70 per cent alcohol from gluten and separated by evaporating to dryness contained 0.29 per cent of phosphorus, but when precipitated from the alcoholic extract by dilute sodium chloride solution it contained only 0.19 per cent.

Glutenin also contained 0.21 per cent of phosphorus. Both these proteins contained iron. Phosphorus and iron are not constituents of the molecules of these proteins, but are derived from the cell nuclei.

Although gliadin and glutenin contain phosphorus, they are not nucleo-proteids. Naysmith also concluded that no ferment action occurred in the formation of gluten.

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[1] Osborne & Harris, Journal American Chemical Society, 1903, XXV, p. 323.
[2] Ibid., p. 474.
[3] Ibid., p. 844.
[4] Ibid., p. 854.
[5] Kutscher, Zeitschrift für physiologische Chemie, 1903, XXXVIII, p. 111.
[6] Naysmith, Transactions of the Canadian Institute, 1903, VII.

The composition of gliadin and glutenin as prepared by Naysmith were as follows:

Composition of gliadin and glutenin as prepared by Naysmith.

	Gliadin.	Glutenin.
	P. ct.	*P. ct.*
Carbon	52.39	52.75
Hydrogen	6.84	7.22
Nitrogen	17 47	16 15
Sulphur	1.12	1.06
Oxygen	21.89	22.58
Phosphorus	0.267	0.215
Iron	0.034	0.026
	100.000	100.000

Snyder[1] proposed a method for determining gliadin in wheat flour which was based on the optical rotation of the alcoholic extract of a definite quantity of the flour. The results of this method agreed closely with those obtained by determining nitrogen in the alcoholic extract. The specific rotation of gliadin calculated from the mean of several determinations by the above method is $(\alpha)_D = -90°$, approximately.

Chamberlain[2] studied the method of determining gliadin and glutenin in wheat flour which had been proposed by Fleurent and modified by Manget, and found that the results for gliadin were too high and for glutenin too low. He therefore proposed a method based on the work of Snyder and of Osborne & Voorhees.

König & Rintelen[3] published an account of their investigation of the proteins of wheat gluten and conclude with Ritthausen that there are three soluble in alcohol. Their analyses of the preparations representing these three proteins agreed closely with those of Ritthausen for gliadin, but differed considerably in carbon for those representing gluten-fibrin and mucedin.

Osborne & Harris[4] reviewed the work that had been done on the alcohol-soluble proteins of wheat published since 1893 and gave their reasons for adhering to the views of Osborne & Voorhees, that only one such protein

[1] Snyder, Journal American Chemical Society, 1904, XXVI, p. 263.
[2] Chamberlain, U. S. Dept. of Agriculture, Bureau of Chemistry, 1904, Bulletin 81.
[3] König & Rintelen, Zeitschrift für Untersuchung der Nährungs und Genussmittel, 1904, VIII, p. 401.
[4] Osborne & Harris, American Journal of Physiology, 1905, XIII, p. 35.

existed, namely, gliadin, the gluten-fibrin and mucedin being, in their opinion, impure preparations of gliadin. This opinion was supported by further experimental evidence and by quantitative determinations of the glutaminic acid yielded by hydrolyzing different fractional preparations of gliadin, some of which, according to Ritthausen's statements, should have contained the gluten-fibrin. The amount of glutaminic acid found in the different fractions was essentially the same.

The glutaminic acid thus obtained from gliadin was 37.3 per cent, which is more than that from any protein substance yet examined.

Abderhalden & Samuely[1] determined the amount of the various primary decomposition products yielded by gliadin and found—

	P. ct.		P. ct.
Glycocoll	0.68	Aspartic acid	1.24
Alanine	2.66	Phenylalanine	2.60
Amino valerianic acid	0.33	Serine	0.12
α-proline	2.40	Tyrosine	2.37
Leucine	6.00	Tryptophane, about	1.00
Glutaminic acid	27.60		

The gliadin used for this analysis yielded 12 per cent of humus, which, together with moisture and ash, were deducted in calculating the above percentages.

Mathewson[2] determined the specific rotation of gliadin in various organic solvents with the following results:

	°
Methyl alcohol, 70 per cent $(a)\frac{40^{\circ}}{D}$	— 95.65
Ethyl alcohol, 70 " " "	— 91.95
Ethyl alcohol, 60 " " "	— 96.66
Ethyl alcohol, 50 " " "	— 98.45
Propyl alcohol, 60 " " "	—101.10
Phenol, 70 " " "	—123.15
Phenol, anhydrous	—131.77
Paracresol	—121.00
Benzyl alcohol	— 53.10
Glacial acetic acid	— 78.60

Osborne & Harris[3] described the preparation of large quantities of the wheat proteins to be used for the quantitative determination of the products of hydrolysis. These determinations were made by Osborne & Clapp.[4] The results described in these papers are given in full later in this publication.

[1] Abderhalden & Samuely, Zeitschrift für physiologische Chemie, 1905, XLVI, p. 193.
[2] Mathewson, Journal American Chemical Society, 1906, XXVIII, p. 1482.
[3] Osborne & Harris, American Journal of Physiology, 1906, XVII, p. 223.
[4] Osborne & Clapp, ibid., p. 231.

EXPERIMENTAL PART.

Although positive evidence of the chemical individuality of protein substances can not yet be obtained, there is no question that protein preparations can be isolated from seeds and animal tissues which, beyond doubt, represent distinctly different substances. Thus five unquestionably different forms of protein, differing in composition, solubility, and physical characters, can be isolated from the wheat kernel. Whether each of these is itself a chemical individual or a mixture of two or more very similar substances can not at present be asserted. All that can be said is that it has not yet been possible to separate them into fractions, the properties of which indicate a mixture.

Owing to the extreme sensitiveness of proteins to the action of acids, alkalis, and salts, the minor differences in solubility are not to be depended upon as a basis for characterizing individual proteins. Thus the protein edestin, which in pure water is entirely insoluble, in the presence of a slight amount of acid is freely soluble therein. The addition of a small quantity of a neutral salt throws the edestin out of this acid solution, while a larger quantity of salt at once redissolves it.

Such differences in solubility have nothing to do with the protein molecule proper, but depend on the formation of protein salts, the solubility of which is different from that of the free protein itself. As the formation of such protein salts depends on conditions that in most cases can not be taken into account, such differences in solubility can not be made a basis for characterizing the different individual proteins.

We are therefore limited, in dealing with such problems, to the more marked differences in solubility, such as that in alcohol, strong saline solutions, or alkalis, and to constant ultimate composition of successive fractional precipitations. Thus, when proteins have been separated into fractions which have the same composition, general solubility, and physical properties, we are not justified in concluding that we have in hand a single individual protein. All we can conclude is that we have reached the limit of separation attainable with the means now available, and that for the present we must accept such products as the simplest units with which we can now deal and which for the present must serve as our basis for further study. If, on the other hand, protein preparations, characterized in the manner above described, show distinct and constant differences from one another, we are justified in considering them to be different substances.

That the wheat kernel contains at least five such distinct protein substances will be shown in the following pages.

THE AQUEOUS EXTRACT OF WHEAT FLOUR.

The water-extract of wheat flour is of a straw-yellow color, becoming red-brown on standing, and has a *very* slight acid reaction towards litmus.

Saturated with ammonium sulphate, a bulky precipitate forms, which on standing contracts, showing the solution to contain but a small amount of protein matter. After 24 hours this precipitate can be completely dissolved in water, giving no evidence of the formation of insoluble derivatives. Saturation with sodium chloride gives a small precipitate. Acetic acid in the cold solution gives no precipitate until sodium chloride is added.

On slowly heating, the solution becomes turbid at 48° and yields a flocculent coagulum at 52°. After heating to 65° for some time and filtering, the solution becomes turbid again at 73°, flocks forming in very small amount at 82°. No more separation occurs on further heating the extract even to boiling. The addition of a little acetic acid and sodium chloride gives a small precipitate. The body coagulating at 52° forms the greater part of the protein in solution. The complete coagulation of this protein is accomplished with difficulty, prolonged heating at 65° being necessary to cause it to separate completely. The addition of sodium chloride greatly facilitates the final coagulation. The temperature at which the flocculent coagulum separates depends upon the rate of heating. Unless the solution is heated very slowly, the point at which flocculation occurs is much above 52°.

When the sodium-chloride extract of the wheat flour is saturated with ammonium sulphate and the precipitate redissolved, and its solution dialyzed until all of the globulin has separated, the solution, when slowly heated in a double water-bath, becomes turbid at 48° and flocks separate at 55°. After heating for some time at 65° and filtering off the coagulum, the solution, when again heated, becomes turbid at 70° and a very minute amount of flocculent coagulum forms at 80°. Boiling the solution after filtering gives no more precipitate, and nothing is obtained by adding a *little* salt and acetic acid. If the amount of salt is increased and acetic acid added, a precipitate results. Equal volumes of a solution so prepared were treated with 20 per cent of sodium chloride and a little acetic acid. To the first the salt and acid were added directly, to the second after heating to 65° and filtering off the coagulum, and to the third after heating to 95° and likewise filtering. The first portion gave the most precipitate, the last the least, showing that the coagulable proteins are thus precipitated by salt and acid. The filtrate from the first portion when neutralized and boiled gave no precipitate, indicating that the separation of the albumin was complete.

The above solution, freed from globulins by dialysis, gave a precipitate on saturation with sodium chloride, the filtrate from which became turbid

when heated to 43°, flocculent at 56°, and no more precipitation on further heating, showing that either a higher-coagulating protein was thus removed or that coagulation of the albumin was more complete in the strong saline solution at the lower temperature. This dialyzed solution likewise gave a considerable precipitate with nitric acid. On heating, a part remained insoluble, and on filtering this off, the filtrate gave a precipitate on cooling, which dissolved again on heating and reappeared as often as the solution was cooled. The filtrate from the salt and acid precipitate did not give this reaction, but the solution of the precipitate in water gave it very strongly.

This reaction is characteristic of some proteoses, and shows that the salt and acid precipitate contains a proteose together with the albumins. This proteose is likewise precipitated by saturating the extract with salt, for on dissolving the precipitate so produced and separating the albumin contained in it by coagulation the filtrate gave a strong *red* biuret reaction, and a precipitate with nitric acid, which dissolved on warming and precipitated again on cooling. The filtrate from the precipitate caused by saturation with salt gives no reaction with nitric acid, showing that the proteose is thus completely precipitated.

In order to be sure that the coagulable protein, which was apparently an albumin, was not a globulin held in solution by the small amount of salts contained in the river-water used for dialysis, as was suggested by its partial precipitation by saturation with sodium chloride, the following experiment was tried: 250 cc. of a strong aqueous extract of winter-wheat meal was dialyzed in running distilled water for 48 hours. A small precipitate was then filtered off, the clear solution returned to the dialyzer, and the process continued for five days longer. No more substance separated. The entire solution, which was still found to coagulate at 54°, was then evaporated to dryness, the considerable protein residue burned off, and the total mineral matter found to weigh only 0.0008 gram, thus proving the protein to be an albumin.

We are able, then, to recognize two distinct protein substances soluble in pure water, namely, a coagulable albumin and a proteose. As it was found that the protein removed from the flour by treatment with alcohol was to a slight extent soluble in pure water, it might be thought that one of these bodies was identical with this alcohol-soluble protein. Its identity with the albumin is excluded by the fact that the latter is precipitated by heat, and with the proteose by the fact that the alcohol-soluble proteose gives a precipitate with hydrochloric acid when it is dissolved in distilled water and also with a *little* sodium chloride, which the proteose does not.

THE ALBUMIN OF WHEAT FLOUR—LEUCOSIN.

A quantity of wheat flour was extracted with 10 per cent sodium-chloride solution, the extract saturated with ammonium sulphate, the precipitated protein dissolved in sodium-chloride brine, and the clear solution dialyzed until all the globulin had been precipitated. The filtered solution was then heated to 60° in a water-bath kept between 60° and 65°. After an hour the precipitate was filtered off, washed thoroughly with water, alcohol, absolute alcohol and ether, and dried. Before drying, the coagulum was a white, voluminous, semisolid mass, which, when completely dried over sulphuric acid, became dense and horny. This preparation, 1, after drying at 110°, was found to have the composition shown in the following table:

Preparations 1 and 2.

	Preparation 1.				Preparation 2.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	53.04	53.28	53.16	53.27	52.91	52.98	52.95	53.06
Hydrogen....	6.74	6.89	6.82	6.83	6.86	6.74	6.80	6.82
Nitrogen.....	16.86	16.95	16.91	16.95	16.93	17.01	16.97	17.01
Sulphur.....	1.27	.....	1.27	1.27	1.28	1.31	1.30	1.30
Oxygen.....	.....	.....	.....	21.68	.....	.....	.....	21.81
Ash.....	0.22	.....	.....	.....	0.22	.....	.....	.....
	.....	.....	.....	100.00	.....	.....	.....	100.00

Another lot of 10,000 grams of flour was extracted with 10 per cent sodium chloride solution and treated in the same way as the extract last described. The albumin obtained weighed 6.4 grams and had the composition given in the above table, preparation 2.

The filtrate from preparation 2 was then heated to 75° and 0.65 gram of coagulum obtained, which on analysis gave the results shown in the table on page 21, preparation 3.

Another preparation was made from the same flour by extracting 10,000 grams with 10 per cent sodium chloride solution, filtering the extract, and dialyzing at once. In this case the precipitation of the proteins with ammonium sulphate was omitted. After complete dialysis the solution was filtered from the globulin which had separated, and the solution heated at once to 90°—a temperature high enough to precipitate all of the albumin.

Dried over sulphuric acid, this preparation, 4, which in all respects resembled 1 and 2, weighed 16.5 grams and when analyzed gave the results shown in the table at the top of page 21.

Preparations 3, 4, and 5.

	Preparation 3.		Preparation 4.				Preparation 5.	
	I.	Ash-free.	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	.....	.....	52.86	.....	52.86	53.02	52.36	52.71
Hydrogen	.....	.....	6.85	.....	6.85	6.87	6.80	6.85
Nitrogen	16.91	16.94	16.21	16.20	16.21	16.26	16.62	16.73
Sulphur	.....	.....	1.20	.....	1.20	1.20	1.34	1.34
Oxygen	.....	.....	.....	.....	.....	22.65	.....	22.37
Ash	0.18	.....	0.32	.....	0.32	.....	0.67	.....
	.....	.....	.....	.....	.....	100 00	.....	100.00

Another preparation of albumin was made by extracting with 10 per cent sodium chloride solution 2000 grams of so-called "shorts" from the spring-wheat flour. This substance consisted chiefly of particles of the outer coats of the seed to which more or less of the adjacent embryo and endosperm adhered. After three hours the extract was strained through a coarse cloth and squeezed out from the residue in a screw-press. After the starch had settled, the nearly clear extract, which had a deep red-brown color, was siphoned off and saturated with ammonium sulphate. The precipitate thus produced was filtered off and dissolved in 10 per cent sodium-chloride brine. The resulting solution, filtered from all the insoluble matter, was then dialyzed free from chlorides, the precipitated globulins filtered off, and the albumin contained in the solution separated by heating to 65°. This preparation, 5, had the composition shown in the preceding table.

TABLE 1.—*Summary of analyses of coagulated wheat albumin.*

	1.	2.	3.	4.	5.	Average.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	53.27	53.06	.....	53.02	52.71	53.02
Hydrogen	6.83	6.82	.....	6.87	6.85	6.84
Nitrogen	16.95	17.01	16.94	16.26	16.83	16.80
Sulphur	1.27	1.30	.....	1.20	1.34	1.28
Oxygen	21.68	21.81	.....	22.65	22.27	22.06
	100.00	100.00	.....	100.00	100.00	100.00

The agreement of the figures in table 1 is satisfactory, with the exception of the nitrogen in 4. The accuracy of this analysis in this respect, however, can not be doubted, as four determinations of this element were made, all of which agreed closely. As this preparation was separated at a higher temperature than any of the others, it is possible that it had in consequence lost some of its nitrogen.

THE AQUEOUS EXTRACT OF THE WHEAT EMBRYO.

The embryo flour, when treated with water, yields a gummy mass, from which a clear extract is secured with difficulty. From 500 grams of meal an extract was obtained with 2000 cc. of water, of which 1400 cc. could be filtered clear. This extract was neutral to litmus, alkaline to lacmoid, and so acid to phenolphthalein that 19 cc. of decinormal alkali were required to neutralize 100 cc. of it to this indicator.

When a freshly prepared dilute aqueous extract of the recently ground wheat germs is heated in a water-bath, no coagulation occurs, the solution becoming slightly opalescent. If a more concentrated extract, such as may be obtained by treating one part of meal with five parts of water, is thus heated, the entire solution solidifies to a firm, opaque jelly, free from visible particles. If to either of these solutions a very little hydrochloric acid is added previous to heating, an abundant flocculent coagulum separates on heating.

After standing a while the aqueous extract becomes gradually acid to litmus, so that when heated slowly it becomes turbid at about 50° and a large flocculent coagulum separates at 55°. Heated to 65° for some time and filtered, a second coagulum may be obtained on raising the heat from 65° to 100°. The amount of this second coagulum is about one-third that of the first.

The coagulated protein is dissolved by 0.5 per cent potassium-hydroxide solution, but not perceptibly by 0.4 per cent hydrochloric acid solution, unless the latter is heated, when a clear transparent jelly is formed.

Freed from coagulable protein, the aqueous extract still contains a relatively large amount of substance which has the reactions of proteose.

When the concentrated aqueous extract is poured into a large volume of distilled water, a turbidity forms at first, which mostly disappears after shaking, indicating the absence of a notable quantity of globulin held in solution by the salts dissolved from the meal.

Saturation of the extracts with sodium chloride gives a considerable precipitate, only a small part of which can be redissolved in dilute sodium-chloride solution. When this dissolved part is precipitated by again saturating with sodium chloride, it also is converted, to a large extent, into an insoluble form ; the part still remaining in solution is precipitated, like a globulin, by dialysis.

When the solution, saturated with sodium chloride, is filtered and the diluted filtrate saturated with ammonium sulphate, a part of the precipitate produced, when redissolved in water, is thrown out of solution by saturating with sodium chloride, though before precipitation with ammonium sulphate it was soluble in saturated sodium-chloride solution.

These reactions show that changes occur which involve the albumin coagulating at 55°, for after freeing the extract from all protein precipitable by saturating with sodium chloride or by dialysis there remains in solution only a small proportion of this albumin.

Thus an aqueous extract corresponding to 666 grams of germ meal, when heated to 65°, yielded 62 grams of coagulum, or 9.3 per cent; a similar extract on dialysis deposited 9.2 per cent; only 0.87 per cent of coagulable and 2.0 per cent of uncoagulable protein remaining in solution. The precipitate, produced by dialysis, was but slightly soluble in sodium chloride solution, having become largely coagulated. From these facts it is clear that one and the same protein substance gives rise to these apparently different protein bodies, and consequently the substance which O'Brien considered to be a globulin of the myosin type and an albumin, coagulating at 80°, are in fact derivatives of the albumin, which coagulates mostly at 65°.

These changes are due to a slow development of acid in the extract, which not only brings about hydrolytic changes in the protein, but may also lead to the formation of different compounds between the protein and the various acids contained in the extract, and so give rise to chemically different substances. Such a development of acid takes place rapidly in muscle plasma, under the influence of which quite similar changes in the proteins there present can be observed.

Why Frankfurt overlooked albumin, present in such large proportion in the aqueous extract, is not easily understood, unless, before heating his solutions, he either added no acid or so much that he converted this substance into an uncoagulable acid compound.

Hydrochloric acid added to the extract in very small quantity causes a flocculent coagulum to separate on heating, while a slightly larger quantity, added before heating, entirely prevents the formation of this coagulum. Acetic acid and nitric acid give precipitates in the extracts which are not soluble in a reasonable excess of either of these acids.

The Albumin of the Wheat Embryo.

In order to determine definitely the relations of these variously obtained substances, a large number of fractional precipitations have been made under quite different conditions, an account of which is now given ·

An extract was made by treating 700 grams of germ meal with 5 liters of water, straining through bolting-cloth and filtering the fluid perfectly clear. A portion of it was at once heated for 1 hour in a water-bath at 60°, and the large coagulum produced gave 24 grams of preparation 6.

Another preparation was made by heating in a water-bath at 65° 2000 cc. of a clear aqueous extract, obtained by treating 3000 grams of the germ meal

with 9 liters of water. The coagulum produced, weighing 62 grams, formed more than 9 per cent of the oil-free germ meal. This is preparation 7.

Another aqueous extract was heated at 65° until all the protein coagulable at this temperature had separated. The coagulum produced, when washed with hot water and alcohol, was dried over sulphuric acid and found to weigh 16.68 grams. The filtrate from this coagulum, heated in a boiling water-bath, yielded a second coagulum which formed preparation 8, weighing 4.9 grams.

A portion of the extract which yielded preparation 6 was saturated with ammonium sulphate; the resulting precipitate was dissolved as far as possible in water, its solution filtered clear and dialyzed for 4 days. During this time a considerable precipitate formed that, when filtered out, was found to be insoluble in salt solution. The solution, filtered from that substance and dialyzed in running water until nothing more separated, was filtered and heated at 60°, which caused a coagulum. This coagulum weighed 7.1 grams and made preparation 9.

When 2000 cc. of an extract of 650 grams of wheat-germ meal was dialyzed 4 days, a dense turbidity formed, due to a globulin, since it dissolved on adding sodium chloride. Passing carbon dioxide gas through the dialyzing solution seemed to increase the turbidity, but effected no definite separation. As it was found that 10 cc. of decinormal hydrochloric acid per 100 cc. of the extract caused a separable precipitate, this proportion was added and the resulting flocculent precipitate brought into solution again by adding sodium chloride. The clear solution was then dialyzed for 2 days in running water and filtered from an amorphous precipitate, which was treated as later described on page 44.

The filtrate from this precipitate was further dialyzed for 3 days more in running water and then, as nothing separated, for 4 days more in alcohol. The precipitate which resulted was dried over sulphuric acid, exhausted with water, in order to remove all uncoagulated proteins, as well as other soluble substances, dehydrated with absolute alcohol, again dried and weighed, yielding 12 grams of preparation 10.

Another aqueous extract was saturated with pure sodium chloride, the abundant precipitate filtered out, treated with dilute brine, and the resulting solution filtered from a relatively considerable quantity of insoluble matter. This filtrate was saturated with sodium chloride, a second precipitate filtered out, and likewise treated with dilute sodium-chloride solution. The insoluble portion was removed by filtration and the clear filtrate dialyzed. The small precipitate separated by dialysis when washed and dried, weighed 4.8 grams and formed preparation 11.

The filtrate from the first precipitation of the substance of preparation 11, caused by saturating its solution with sodium chloride, as described above, was diluted with water and saturated with ammonium sulphate. The precipitate which resulted was dissolved in water and its solution precipitated by saturating with sodium chloride. Although this substance had previously been soluble in saturated brine, after precipitation with ammonium sulphate it was found to be nearly all insoluble therein, so that almost complete precipitation resulted on again saturating with sodium chloride. The precipitate so produced was filtered out, dissolved in dilute sodium chloride solution, and reprecipitated by dialysis. We thus secured 7.6 grams of preparation 12.

By saturating another aqueous extract of germ meal with sodium chloride a very large quantity of protein was separated, which was filtered out, exhausted with dilute sodium chloride solution, and the insoluble part washed thoroughly with water and alcohol. This preparation, 13, weighed 17 grams.

The filtrate and saline washings from preparation 13 were united and again saturated with sodium chloride and yielded a small precipitate, which, when dissolved in brine and precipitated by dialysis, gave preparation 14, weighing 2.8 grams. As the salt-saturated solution from which this preparation had separated contained so little protein, it appears that nearly all the protein precipitated from the aqueous extract by saturating with sodium chloride had been converted into the insoluble substance forming preparation 13.

The filtrate from the salt-saturated precipitate produced in the aqueous extract was dialyzed in water for several days, and the still clear solution then dialyzed in alcohol for 24 hours. The protein thereby precipitated in a coagulated state yielded 12.4 grams of preparation 15.

Another aqueous extract was saturated with sodium chloride, and the precipitate, treated in the same way as preparation 13, yielded 18 grams of preparation 16.

The saline washings of the last preparation were dialyzed free from chlorides and gave a precipitate weighing 2.86 grams, which formed preparation 17, having the properties of a globulin, dissolving readily on adding sodium chloride, and being precipitated from such solution by water.

The filtrate from the final precipitation of 17, when heated in a boiling water-bath, gave a coagulum, which formed preparation 18, weighing 1.64 grams.

The salt-saturated filtrate from the first precipitation of 16, as already described, was heated to boiling, and the coagulum produced was filtered out, giving preparation 19, weighing 5.47 grams.

Since analysis showed that most of the preparations already described contained phosphorus, some even in large amount, we made an attempt to

separate the phosphorus from our extract in order to determine, if possible, the relation of the preparations free from phosphorus to those which contained much phosphorus.

2000 grams of meal were treated with 6 liters of distilled water, and the extract (4 liters) was squeezed out as completely as possible in a press.

As a preliminary experiment 100 cc. of this clear, filtered extract were made faintly alkaline to phenolphthalein, with about 40 cc. of decinormal potassium-hydroxide solution. To insure a sufficient quantity 20 cc. more of alkali were added, and thereupon a little calcium chloride, which gave a precipitate that seemed to partly dissolve on adding sodium chloride. The undissolved part, when washed with dilute sodium-chloride solution, water, and alcohol, and dried, formed 1.7 per cent of the meal, contained about 55 per cent of organic matter, and left 45 per cent of ash, consisting of tricalcium phosphate.

To 2000 cc. of the original extract were then added 1350 cc. of a solution containing alkali, equivalent to 1560 cc. decinormal solution, with sodium chloride enough to form 6.5 per cent of the total liquid. To this a solution of calcium chloride was added as long as a precipitate formed, and after standing over night the solution was decanted from the precipitate and filtered clear on a pulp filter. Of the clear filtrate 2200 cc. were made as neutral as possible to litmus by adding 180 cc. of decinormal hydrochloric acid solution. Of the solution thus neutralized 1000 cc., when gradually heated in a water-bath, became turbid at 52° and a considerable coagulum separated at 53°.

After the temperature had been slowly raised to 65° and kept at this point some time, the coagulum was filtered out, washed, and dried as usual, giving preparation 20, weighing 6.4 grams. Another portion of this extract, filtered from the calcium-chloride precipitate, was saturated with ammonium sulphate while still slightly alkaline to litmus, the resulting precipitate filtered out, dissolved in water, its solution filtered clear, and dialyzed. A slight precipitate formed on dialysis, which was removed by filtering and the solution heated in a boiling water-bath. The protein thus coagulated weighed 3.07 grams, preparation 21.

To determine what effect the removal of the phosphorized substance thrown out by calcium chloride had upon the precipitation with sodium chloride, we made neutral to litmus a liter of the filtrate from the calcium-chloride precipitate and then saturated it with sodium chloride. The large precipitate which formed was washed by decantation with water, in which it gradually dissolved, until only an insignificant quantity remained. The similarly obtained precipitate from the simple aqueous extract we have shown to be nearly all insoluble in water.

To separate globulin from the aqueous extract 1200 cc. of clear, filtered extract were obtained from 200 grams of the germ meal treated with 2000 cc. of water. 1000 cc. of this extract were dialyzed in running water for 6 days, and the large precipitate resulting gave preparation 22, weighing 9.17 grams.

These preparations, thus variously obtained from the aqueous extract, were dried to constant weight at 110° and analyzed with the results given in table 2, most of the figures being the average of closely agreeing duplicate determinations.

TABLE 2.—*Composition of preparations of protein from the water extract of the wheat embryo.*

	6.	7.	8.	9.	10.	11.	12.	13.	14.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	51.13	50.52	50.17	52.39	51.77	52.13	52.73	43.59	52.28
Hydrogen ...	6.85	6.81	7.01	6.83	6.81	7.04	7.11	5.77	6.97
Nitrogen	16.28	16.47	16.66	16.20	16.11	16.48	16.00	15.16	16.38
Sulphur	1.18	1.17	1.00	1.32	1.30	1.49	1.53	0.90	1.39
Phosphorus..	0.72	0.97	0.91	trace	0.17	0.06	none	3.38	0.07
Ash	2.73	2.90	3.03	0.35	1.39	0.43	0.39	13.04	0.44
P_2O_5 in ash ..	1.83	2.09	1.91	trace	0.47	trace	none	6.73	trace

	15.	16.	17.	18.	19.	20.	21.	22.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	51.21	46.67	51.87	.....	51.91	51.65	52.02	49.59
Hydrogen	6.85	6.19	6.89	.....	6.86	6.66	7.00	6.68
Nitrogen.....	16.18	15.89	16.65	16.31	16.08	16.02	16.45	16.34
Sulphur	1.10	0.93	1.19	1.35	1.60	1.13	1.24	0.91
Phosphorus	0.46	2.53	trace	trace	trace	trace	none	1.85
Ash	2.19	8.17	0.38	0.45	0.32	1.09	0.56	2.50
P_2O_5 in ash.....	1.11	5.71	trace	trace	trace	trace	none	1.79

Assuming that those of the foregoing preparations which contain phosphorus are compounds of protein with the nucleic acid which has been separated from the aqueous extract of wheat germs,[1] and also assuming that all the phosphorus of these preparations is a part of the nucleic acid, the composition of these preparations was calculated free from nucleic acid. The analyses were further calculated ash-free by subtracting the P_2O_5 contained in the ash from the total ash, which seems permissible, since the ash consisted almost wholly of metaphosphates of potassium and sodium,

[1] Osborne & Campbell, Journal American Chemical Society, 1900, XXII, p. 379; also Osborne & Harris, Report Connecticut Agricultural Experiment Station for 1901, p. 365.

separate the phosphorus from our extract in order to determine, if possible, the relation of the preparations free from phosphorus to those which contained much phosphorus.

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After the temperature had been slowly raised to 65° and kept at this point some time, the coagulum was filtered out, washed, and dried as usual, giving preparation 20, weighing 6.4 grams. Another portion of this extract, filtered from the calcium-chloride precipitate, was saturated with ammonium sulphate while still slightly alkaline to litmus, the resulting precipitate filtered out, dissolved in water, its solution filtered clear, and dialyzed. A slight precipitate formed on dialysis, which was removed by filtering and the solution heated in a boiling water-bath. The protein thus coagulated weighed 3.07 grams, preparation 21.

To determine what effect the removal of the phosphorized substance thrown out by calcium chloride had upon the precipitation with sodium chloride, we made neutral to litmus a liter of the filtrate from the calcium-chloride precipitate and then saturated it with sodium chloride. The large precipitate which formed was washed by decantation with water, in which it gradually dissolved, until only an insignificant quantity remained. The similarly obtained precipitate from the simple aqueous extract we have shown to be nearly all insoluble in water.

To separate globulin from the aqueous extract 1200 cc. of clear, filtered extract were obtained from 200 grams of the germ meal treated with 2000 cc. of water. 1000 cc. of this extract were dialyzed in running water for 6 days, and the large precipitate resulting gave preparation 22, weighing 9.17 grams.

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Hydrogen ...	6.85	6.81	7.01	6.83	6.81	7.04	7.11	5.77	6.97
Nitrogen	16.28	16.47	16.66	16.20	16.11	16.48	16.00	15.16	16.38
Sulphur	1.18	1.17	1.00	1.32	1.30	1.49	1.53	0.90	1.39
Phosphorus..	0.72	0.97	0.91	trace	0.17	0.06	none	3.38	0.07
Ash	2.73	2.90	3.03	0.35	1.39	0.43	0.39	13.04	0.44
P_2O_5 in ash ..	1.88	2.09	1.91	trace	0.47	trace	none	6.73	trace

	15.	16.	17.	18.	19.	20.	21.	22.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	51.21	46.67	51.87	.....	51.91	51.65	52.02	49.59
Hydrogen	6.85	6.19	6.89	.....	6.86	6.66	7.00	6.68
Nitrogen.....	16.18	15.89	16.65	16.31	16.08	16.02	16.45	16.34
Sulphur	1.10	0.93	1.19	1.35	1.60	1.13	1.24	0.91
Phosphorus.....	0.46	2.53	trace	trace	trace	trace	none	1.85
Ash	2.19	8.17	0.38	0.45	0.32	1.09	0.56	2.50
P_2O_5 in ash.....	1.11	5.71	trace	trace	trace	trace	none	1.79

Assuming that those of the foregoing preparations which contain phosphorus are compounds of protein with the nucleic acid which has been separated from the aqueous extract of wheat germs,[1] and also assuming that all the phosphorus of these preparations is a part of the nucleic acid, the composition of these preparations was calculated free from nucleic acid. The analyses were further calculated ash-free by subtracting the P_2O_5 contained in the ash from the total ash, which seems permissible, since the ash consisted almost wholly of metaphosphates of potassium and sodium,

[1] Osborne & Campbell, Journal American Chemical Society, 1900, XXII, p. 379; also Osborne & Harris, Report Connecticut Agricultural Experiment Station for 1901, p. 365.

strongly indicating that the P_2O_5 was derived from the nucleic acid. These calculations gave the results shown in table 3.

TABLE 3.—*Composition of leucosin contained in the preparations from water extracts of the wheat embryo.*

	6.	7.	8.	9.	10.	11.	12.	13.	14.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.93	52.75	52.41	52.57	52 57	52.47	52.93	53.23	52.64
Hydrogen	7.12	7.16	7.38	6.85	6.91	7.08	7.13	7.09	7.02
Nitrogen	16.45	16.68	16.94	16 26	16.27	16.55	16 06	16.30	16.46
Sulphur	1.29	1.32	1.13	1.32	1.34	1.50	1.53	1.60	1.41
Oxygen	22.21	22.09	22.14	23.00	22.91	22.40	22.35	21.78	22.47
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	15.	16.	17.	18.	19.	20.	21.	22.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.63	52.44	52.06	.....	52.11	52.16	52.30	53.45
Hydrogen	7.06	7.10	6.92	.....	6.88	6.73	7.04	7.30
Nitrogen	16.40	16.26	16.71	16.38	16.13	16.20	16.54	16.57
Sulphur	1.17	1.34	1.19	1.35	1.60	1.14	1.24	1.16
Oxygen	22.74	22.86	23.12	.....	23.28	23.77	22.88	21.52
	100.00	100.00	100.00	.....	100.00	100.00	100.00	100.00

Of these preparations, 6, 7, 8, 9, 18, 19, 20, and 21 were obtained by coagulation with heat, 10 and 15 by coagulation with alcohol, 13 and 16 by saturation with sodium chloride, 11, 12, 14, and 17 by dialyzing salt solutions in water, and 22 by direct dialysis of the aqueous extract. Since some of these preparations formed a large part of the protein contained in the extract, while others represented fractions of it, it is evident that all contain protein of the same composition, mostly combined with various proportions of nucleic acid.

Eliminating the nucleic acid, it thus appears that the composition of the protein part of those preparations which contain phosphorus is the same as that of the phosphorus-free protein preparations, although the former contain from very little up to more than 37 per cent of nucleic acid.

Most of these preparations might, in accordance with custom, be called nucleoproteids, while 13 and 16 are, both in properties and composition, very much like nuclein. It is probable that these nucleoproteids and nucleins are nucleic acid compounds of one and the same protein.

It is to be noted that these preparations show very diverse properties. Some are like albumin; some like globulin; some are precipitated by satura-

tion with salt, while others are not. As we have shown, these different properties are the result of changes caused by varying the conditions under which the protein exists in the extract, and depend chiefly on the degree of acidity of the extract, whereby the numbers and kinds of acid molecules that combine with the protein molecule are altered.

Whatever may be the true cause of these changes, it is evident from the results here described that the distinctions heretofore made between globulin and albumin, myosin, and vitellin, etc., have very little value as a basis for classifying protein substances. This explains the difference between O'Brien's classification of leucosin as a myosin-like globulin, to which reference was made at the beginning of this paper, and our designation of it as an albumin, because of the ready solubility in water and coagulability by heat of the preparations which we had made.

Thus preparation 22, weighing 9.17 grams, was insoluble in water and in salt solution, and was not a precipitate of globulin, since in the filtrate from which it had separated on dialysis only 0.87 gram of coagulable albumin was found instead of 9.5 grams, as usually found by direct coagulation of the aqueous extracts; moreover, the analysis indicates that it is a compound of leucosin, with 20 per cent of nucleic acid.

On the preceding pages it was shown that a small part of the precipitate, produced by saturating the aqueous extract with sodium chloride, is soluble in dilute salt solution, and can be precipitated from this solution by dialysis, as a globulin-like substance, readily soluble again in salt solution. This globulin-like substance contains little or no nucleic acid, and has very nearly the same elementary composition as leucosin, of which it is probably a compound with a small proportion of some body of low molecular weight.

It is plain from these facts that O'Brien's myosin contains the same protein substance as my leucosin.

O'Brien's "albumin," coagulating at 75° to 80°, is probably more of this same leucosin, as shown by preparation 8, which formed about 25 per cent of the total coagulable protein. The experience of the writer has been that complete coagulation, especially in a solution nearly free from salts, can be effected, if at all, only by heating the solution much above the lower coagulation temperature of the protein to be separated.

From the whole seed leucosin was obtained with the same composition and general properties as from the embryo, but preparations from the whole seed were free from phosphorus. This was probably because the proportion of nucleic acid to protein matter was smaller in the whole seed than in the embryo, so that on extracting with water the nucleic acid did not form soluble compounds with the leucosin, but remained undissolved in combination with the other proteins. In table 4, on the following page, is given the average of analyses of albumin from the cereals.

TABLE 4.—*Composition of albumin prepared from various cereals.*

	Wheat embryo.	Wheat kernel.	Rye kernel.	Barley kernel.	Barley malt.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.65	53.02	52.97	52.81	53.07
Hydrogen	7.04	6.84	6.79	6.78	6.72
Nitrogen	16.43	16.80	16.66	16.62	16.71
Sulphur	1.32	1.28	1.35	1.47	23.50
Oxygen	22.56	22.06	22.23	22.32	
	100 00	100.00	100.00	100.00	100.00

In an earlier paper on the "Chemical Nature of Diastase"[1] I pointed out that diastatic action appeared to be always associated with leucosin. Since extracts of wheat embryo were so rich in leucosin, the diastatic power of the germ meal was determined by extracting with four times its weight of water, and, under the conditions of Litner's test, 0.10 cc. of the extract so made, when added to 10 cc. of a 2 per cent solution of soluble starch, formed within 1 hour, at 20°, enough sugar to reduce 5 cc. of Fehling's solution. The 0.10 cc. of extract corresponds to 25 mg. of the germs, from which it is seen that this meal possesses high diastatic power, though it is inferior in this respect to active malt.

HYDROLYSIS OF LEUCOSIN.

In order to determine the proportion of the different products which are formed by boiling proteins with strong acids, it is necessary to use relatively large amounts of the protein. Commercial wheat-germ meal was therefore used in order to obtain a sufficient quantity of leucosin for this purpose. The freshly ground meal was extracted with water, and, as the gummy solution could not be filtered within a reasonable time, an equal volume of saturated ammonium sulphate solution was added. The precipitate thus produced was filtered out, dissolved in water, the solution filtered perfectly clear, and the leucosin coagulated by heating the dilute solution to 65° in a water-bath at 70°. As the only other protein substance present in this solution was a relatively insignificant quantity of proteose, the product obtained was practically free from any other protein. This coagulum was thoroughly washed with hot water, in order to remove any admixed proteose, and dehydrated with absolute alcohol. The preparation formed a light white powder.

Owing to the difficulty of preparing large quantities of this protein, which occurs in very small quantity in the wheat kernel, we were limited in this

[1] Osborne, Journal American Chemical Society, 1895, XVII, p. 587.

hydrolysis to 257.8 grams of water and ash-free leucosin. This was hydrolyzed and the glutaminic acid separated as hydrochloride. When dried in vacuo over sulphuric acid, 12.39 grams of the hydrochloride were obtained, which melted at 198° with effervescence.

Nitrogen: 0.5482 gram substance gave NH_3 = 4.17 cc. HCl (1 cc. HCl = 0.01 gram N).
Chlorine: 0.2528 gram substance gave 0.1989 gram AgCl.
Calculated for $C_5H_{10}O_4NCl$, N 7.64, Cl 19.35 p. ct.; found, N 7.61, Cl 19.45 p. ct.

The filtrate from the glutaminic acid hydrochloride was concentrated to a sirup under reduced pressure, the residue taken up in alcohol and saturated with dry hydrochloric acid gas. The solution was then evaporated to a thick sirup under reduced pressure, the residue again esterified with alcohol and hydrochloric acid, and the solution concentrated as before. The esterification was again repeated, the final concentration being made at a pressure of 10 mm. from a bath, the temperature of which did not rise above 40°. The free esters of the amino-acids were then liberated from the residue, extracted with ether, and dried with potassium carbonate and anhydrous sodium sulphate in the usual way. The aqueous layer was then made strongly acid with hydrochloric acid and the salts removed by concentration and treatment with alcoholic hydrochloric acid. The alcoholic extracts containing the hydrochlorides of the amino-acids were evaporated to a thick sirup under reduced pressure and the residue esterified as above described. The free esters were then liberated and their ether solution dried as before. After distilling off the ether on the water-bath, at atmospheric pressure, the residue was distilled with the following results:

Fraction.	Temperature of bath up to—	Pressure.	Weight.
	°	*mm.*	*Grams.*
I	75	12.0	21.76
II	80	10.0	25.86
III	125	0.8	49.60
IV	200	0.8	49.39
			146.61

{ Fraction I. Temperature of bath up to 75°. Pressure, 12 mm. Weight, 21.76 grams. }

This fraction was saponified directly after collection by evaporating on the water-bath with concentrated hydrochloric acid. The residue was esterified with alcohol and hydrochloric acid and allowed to stand for several days on ice. The glycocoll ester hydrochloride which separated weighed 1.73 grams and melted at 145°.

Nitrogen: 0.2851 gram substance, dried in vacuo, gave $NH_3 = 2.88$ cc. HCl (1 cc. HCl = 0.01 gram N).

Chlorine: 0.2843 gram substance, dried in vacuo, gave 0.2889 gram AgCl.

Calculated for $C_4H_{10}O_2NCl$, N 10.05, Cl 25.40 p. ct.; found, N 10.12, Cl 25.12 p. ct.

In the filtrate from the glycocoll ester hydrochloride the free amino-acids were regenerated and subjected to fractional crystallization in water and alcohol. There were obtained 3.06 grams of nearly pure alanine.

Carbon and hydrogen: 0.3682 gram substance gave 0.5460 gram CO_2 and 0.2649 gram H_2O.

Nitrogen: 0.2508 gram substance gave $NH_3 = 3.92$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_3H_7O_2N$, C 40.40, H 7.93, N 15.75 p. ct.; found, C 40.44, H 7.99, N 15.63 p. ct.

The alanine decomposed at 290°.

{ Fraction II. Temperature of bath up to 80°.
Pressure, 10 mm. Weight, 25.86 grams. }

After saponifying with boiling water, the solution of the amino-acids was evaporated to dryness under reduced pressure. The dried residue, which weighed 18.78 grams, was extracted with boiling alcohol, whereby 1.68 grams went into solution. From the part insoluble in alcohol there were isolated, by fractional crystallization, 5.53 grams of leucine, 5.79 grams alanine, and 0.47 gram of substance which had the percentage composition of amino-valerianic acid.

Carbon and hydrogen: 0.2094 gram substance gave 0.3913 gram CO_2 and 0.1793 gram H_2O.

Calculated for $C_5H_{11}O_2N$, C 51.22, H 9.48 p. ct.; found, C 50.96, H 9.51 p. ct.

From the more soluble part of this fraction glycocoll was isolated as the hydrochloride of the ester. This weighed 2.78 grams, equivalent to 1.50 grams of glycocoll, and melted at 144° to 145°.

{ Fraction III. Temperature of bath up to 125°.
Pressure, 0.8 mm. Weight, 49.6 grams. }

Fraction III was saponified by boiling with 10 parts of water for 10 hours, and the solution evaporated to dryness under reduced pressure. After extracting the proline with boiling alcohol the insoluble part was fractionally crystallized. There were obtained 23.72 grams of leucine and 2.62 grams of alanine. The isolated leucine decomposed at 298°.

Carbon and hydrogen: 0.1835 gram substance dried at 110° gave 0.3670 gram CO_2 and 0.1670 gram H_2O.

Nitrogen: 0.3086 gram substance gave $NH_3 = 3.34$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_6H_{13}O_2N$, C 54.89, H 10.01, N 10.70 p. ct.; found C 54.63, H 10.11, N 10.82 p. ct.

The combined alcoholic solutions from fractions II and III, which contained the proline, were evaporated to dryness under reduced pressure, and the residue extracted with boiling alcohol, in which 2.3 grams did not dissolve. The solution filtered from this was again evaporated to dryness under reduced pressure, the residue dissolved in water, and the copper salts prepared by boiling for an hour with an excess of copper hydroxide. The deep blue solution was evaporated to dryness under reduced pressure, and the residue boiled with absolute alcohol, which dissolved the *l*-proline copper salt. The residue of racemic copper salt, insoluble in alcohol, was dissolved in water and the solution concentrated. Of racemic proline copper 2.41 grams were obtained, equivalent to 1.69 grams of *α*-proline.

Water: 0.3190 gram substance, air-dried, lost 0.0353 gram H_2O at 110°.
Copper: 0.2827 gram substance, dried at 110°, gave 0.0766 gram CuO.
Calculated for $C_{10}H_{16}O_4N_2Cu$. 2 H_2O, H_2O 11.00 p. ct.; found, H_2O 11.07 p. ct.
Calculated for $C_{10}H_{16}O_4N_2Cu$, Cu 21.79 p. ct.; found, Cu 21.65 p. ct.

The alcoholic solution of the copper salt of the *l*-proline was evaporated to dryness under reduced pressure. The dried residue weighed 8.24 grams, equivalent to 6.5 grams of *l*-proline.

For identification a small portion was freed from copper with hydrogen sulphide, and the free proline converted into the phenylhydantoin, according to the directions of Emil Fischer.[1]

The hydantoin melted sharply at 143° and gave the following analysis:

Carbon and hydrogen: 0.2334 gm. substance gave 0.5676 gm. CO_2 and 0.1204 gm. H_2O.
Nitrogen: 0.1373 gram substance gave NH_3 = 1.78 cc. HCl (1 cc. HCl = 0.01 gram N).
Calculated for $C_{12}H_{12}O_2N_2$, C 66.60, H 5.61, N 12.99 p. ct.; found, C 66.32, H 5.73, N 12.96 p. ct.

{ Fraction IV. Temperature of bath up to 200°.
Pressure, 0.8 mm. Weight, 49.39 grams. }

From fraction IV the ester of phenylalanine was removed in the usual manner by shaking out with ether, and after freeing from ether the residual ester was saponified by dissolving in concentrated hydrochloric acid and evaporating on the water-bath. Of phenylalanine hydrochloride 12.12 grams were obtained, which is equivalent to 9.93 grams of phenylalanine. For identification the hydrochloride was recrystallized from strong hydrochloric acid and converted into the free acid by evaporation with excess of ammonia. When once recrystallized from water, it melted at 263° to 265°.

Carbon and hydrogen: 0.2962 gram substance, dried at 110°, gave 0.7089 gram CO_2 and 0.1801 gram H_2O.
Nitrogen: 0.1924 gram substance gave NH_3 = 1.65 cc. HCl (1 cc. HCl = 0.01 gram N).
Calculated for $C_9H_{11}O_2N$, C 65.39, H 6.73, N 8.50 p. ct.; found, C 65.27, H 6.76, N 8.57 p. ct.

[1] Fischer, E., Zeitschrift für physiologische Chemie, 1901, XXXIII, p. 251.

The aqueous layer was saponified by warming with an excess of barium hydroxide on the water-bath for 7 hours. After standing for some time the crystals of racemic barium aspartate were filtered out and decomposed with an equivalent quantity of sulphuric acid. The filtrate from the barium sulphate gave, on concentration, 5.06 grams of aspartic acid, which, when recrystallized from water, was analyzed.

Carbon and hydrogen: 0.2483 gram substance, dried at 110°, gave 0.3278 gram CO_2 and 0.1215 gram H_2O.

Nitrogen: 0.2769 gram substance gave $NH_3 = 2.93$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_4H_7O_4N$, C 36.06, H 5.31, N 10.55 p. ct.; found, C 36.00, H 5.44, N 10.59 p. ct.

The filtrate from barium aspartate was freed from barium quantitatively with sulphuric acid and the filtrate from the barium sulphate concentrated to small volume and saturated with hydrochloric acid gas. After long standing on ice, 2.15 grams of glutaminic acid hydrochloride separated.

The filtrate from this was evaporated under reduced pressure, the residue taken up in water, and the chlorine removed with silver sulphate.

After removing the sulphuric acid with an equivalent quantity of barium hydroxide, the solution was boiled with an excess of copper hydroxide. The filtered solution, on standing, separated tyrosine-like needles of copper lævo-aspartate which weighed 7.41 grams, equivalent to 3.57 grams of aspartic acid.

Nitrogen: 0.1853 gram substance, air-dried, gave $NH_3 = 0.93$ cc. HCl (1 cc. HCl = 0.01 gram N).

Copper: 0.1677 gram substance, air-dried, gave 0.0491 gram CuO.

Calculated for $C_4H_5O_4N$, Cu · 4½ H_2O, N 5.09, Cu 23.06 p. ct.; found, N 5.02, Cu 23.37 p. ct.

From the copper salt the free acid was regenerated and analyzed.

Carbon and hydrogen: 0.2041 gram substance gave 0.2715 gram CO_2 and 0.1054 gram H_2O.

Calculated for $C_4H_7O_4N$, C 36.06, H 5.31 p. ct.; found, C 36.29, H 5.74 p. ct.

Specific rotation.—Dissolved in 20 per cent hydrochloric acid,

$$(\alpha)\ \frac{20^\circ}{D} = +23.8^\circ$$

Fischer & Dörpinghaus[1] found

$$(\alpha)\ \frac{20^\circ}{D} = +22.0^\circ$$

An effort to isolate serine in the filtrate from the copper aspartate failed.

[1] Fischer & Dörpinghaus, Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 462.

The Residue after Distillation.

The residue remaining after distillation of the esters weighed 57 grams. This was dissolved in boiling alcohol, and after cooling 1.98 grams of needle crystals were filtered out. The filtrate was evaporated to a sirup under reduced pressure, saponified by heating with an excess of barium hydroxide, and, after removing the barium, evaporated to small volume under reduced pressure. The solution was then saturated with hydrochloric acid, and, after standing on ice for a long time, yielded 7.39 grams of glutaminic acid hydrochloride. The free acid prepared by evaporating with an exactly equivalent quantity of potassium hydroxide, when recrystallized from water, melted at 202° to 203° with effervescence.

Carbon and hydrogen: 0.3646 gram substance, dried at 110°, gave 0.5432 gram CO_2 and 0.2019 gram H_2O.

Nitrogen: 0.3696 gram substance gave $NH_3 = 3.55$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_5H_9O_4N$, C 40.82, H 6.12, N 9.52 p. ct.; found, C 40.63, H 6.15, N 9.60 p. ct.

The total glutaminic acid obtained from leucosin was 17.5 grams, or 6.73 per cent. This result is higher than that recently recorded in this laboratory, namely, 5.72.[1]

This protein is one from which the glutaminic acid hydrochloride can be directly obtained only with great difficulty. In the former paper attention was directed to this fact, and the statement made that it is possible that the result given was too low.

Tyrosine.

Forty grams of leucosin, equal to 34.96 grams dried at 110°, were boiled for 12 hours with a mixture of 120 grams of sulphuric acid and 240 grams of water. After removing the sulphuric acid with an equivalent quantity of barium hydroxide, the solution was evaporated with an excess of barium carbonate in order to remove ammonia. After removing the barium, the solution was concentrated to a small volume on the water-bath and allowed to stand for some time. The substance which separated was washed with cold water, dissolved in ammonia, the solution treated with bone-black, and evaporated. On cooling, 1.0360 grams tyrosine separated in colorless needles. The filtration from this, on further concentration, yielded 0.13 gram more tyrosine, making a total of 1.1660 grams, or 3.33 per cent. This was recrystalized and analyzed.

Carbon and hydrogen: 0.4573 gram substance, dried at 110°, gave 0.9994 gram CO_2 and 0.2813 gram H_2O.

Calculated for $C_9H_{11}O_3N$, C 59.62, H 6.13 p. ct.; found, C 59.60, H 6.11 p. ct.

[1] Osborne & Gilbert, American Journal of Physiology, 1906, xv, p. 333.

ARGININE, HISTIDINE, AND LYSINE.

The filtrate from the tyrosine, by Kossel's method, yielded a solution in which the nitrogen found corresponded to 0.99 gram of histidine, or 2.83 per cent.

The identity of the histidine could not be established owing to its very small amount.

The solution of the arginine contained nitrogen equal to 0.6720 gram, which is equal to 2.08 grams of arginine, or 5.94 per cent. A part was converted into the nitrate and this latter into the copper salt by boiling with an excess of copper hydroxide and the copper salt recrystallized from water.

Water: 0.1984 gram substance, air-dried, lost 0.0191 gram H_2O at 110°.
Copper: 0.1766 gram substance, dried at 110°, gave 0.0262 gram CuO.
Calculated for $C_{12}H_{28}O_4N_8Cu\ (NO_3)_2 \cdot 3\ H_2O$, H_2O 9.15 p. ct.; found, H_2O 9.62 p. ct.
Calculated for $C_{12}H_{28}O_4N_8Cu\ (NO_3)_2$, Cu 11.85 p. ct.; found, Cu 11.83 p. ct.

The filtrate from the copper salt was freed from copper and the solution evaporated with an excess of sulphuric acid under reduced pressure. The sulphuric acid was removed with an excess of barium hydroxide, and the barium with carbonic acid. The filtrate from the barium carbonate was evaporated to dryness and the arginine converted into the picrolonate, according to the directions of Steudel. This melted at 226° to 227°; Steudel gives 225°.

Nitrogen: 0.0516 gram substance, dried at 110°, gave 11.8 cc. moist N_2 at 25° and 765 mm.
Calculated for $C_6H_{14}O_2N_4 \cdot C_{10}H_8O_5N_4$, N 25.62 p. ct.; found, N 25.71 p. ct.

The filtrate from the silver precipitate which contained the arginine and histidine was freed from silver and barium, and the lysine precipitated with phosphotungstic acid and then converted into the picrate, of which 2.47 grams, equal to 0.9616 gram of lysine, or 2.75 per cent, was obtained. This was recrystallized from water and analyzed.

Nitrogen: 0.2288 gram substance gave 38.6 cc. moist N_2 at 25.5° and 759 mm.
Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3$, N 18.70 p. ct.; found, N 18.77 p. ct.

The results of this hydrolysis are given in table 5.

TABLE 5.—*Leucosin.*

	P. ct.		P. ct.
Glycocoll	0.94	Tyrosine	3.34
Alanine	4.45	Lysine	2.75
Aminovalerianic acid	0.18	Histidine	2.83
Leucine	11.34	Arginine	5.94
α-proline	3.18	Ammonia	1.41
Phenylalanine	3.83	Tryptophane	present
Aspartic acid	3.35		
Glutaminic acid	6.73		50.32

PROTEINS OF WHEAT FLOUR SOLUBLE IN SODIUM-CHLORIDE SOLUTION.

The Globulin of Wheat Flour.

Beside the proteins soluble in water, 10 per cent sodium-chloride brine extracts from ground wheat kernels a globulin which is present in the seed in small quantity. Ten kilograms of "straight flour" were extracted with 34 liters of 10 per cent sodium-chloride solution by suspending the flour in the liquid, stirring frequently, and then allowing the whole to stand at rest over night. The extract, separated from the flour and filtered as clear as possible, had a very slight acid reaction, was of a pink color, very viscid consistence, and formed about one-half of the solution added to the flour. This was saturated with ammonium sulphate, and the resulting precipitate filtered off and dissolved as far as possible in 4 liters of 10 per cent sodium-chloride solution. The exceedingly viscid solution was filtered with difficulty, placed in a dialyzer, and left in a stream of running water until the chlorides were removed. As the salts dialyzed out the globulin gradually separated in minute particles, the larger of these being evidently spheroidal in form. This precipitate weighed 5.8 grams. This protein, dissolved in a 10 per cent sodium-chloride solution, when heated slowly, gave a very slight turbidity at 87°, which increased slightly up to 99°. On boiling, some coagulum developed, and on adding acid to the solution filtered from this coagulum a very considerable precipitate formed.

Dilution of the solution of the globulin in 10 per cent sodium-chloride brine precipitated the protein. Saturation with sodium chloride gave no precipitate. Saturation with magnesium sulphate, and also with ammonium sulphate, completely precipitated the globulin. When dried at 110°, this preparation, 23, gave, when analyzed, the results which are shown in the table on page 38.

Another preparation, 24, was made in the same way as the preceding, except that the precipitation with ammonium sulphate was omitted, the filtered extract being placed at once in dialyzers. Like the preceding solution, this was at first very viscid, but after the removal of the chloride the viscid property was entirely lost. This viscidity can hardly be due to the globulin, for solutions of the precipitated globulin showed no trace of it. The aqueous extract of the flour had no such property, and it is difficult to say to what this was due unless to the presence of carbohydrate. After complete removal of the chlorides the solution was filtered from the precipitate, the latter dissolved in 10 per cent sodium-chloride solution, and the insoluble matter filtered off. The residue so removed consisted chiefly of an insoluble form of the globulin. This was dissolved in 0.2 per cent potassium-hydroxide water, the solution filtered clear, and precipitated by neutralization with

0.2 per cent hydrochloric acid. The precipitate weighed 2 grams. The solution of the globulin was dialyzed till free from chlorides and the separated globulin found to weigh 5 grams. The yield in this extraction was therefore 7 grams, or nearly the same as in the preceding. The composition of preparation 24 is given by the analysis shown in the following table :

Preparations 23 and 24.

	Preparation 23.				Preparation 24.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	50.87	50.79	50.83	51.07	50.77	50.63	50.70	51 01
Hydrogen....	6.74	6.67	6.71	6.75	7.03	6.84	6.93	6.97
Nitrogen.....	18.13	18.25	18.19	18.27	18.43	18.32	18.38	18.48
Sulphur	0.97	.....	0.97	0.97	0.71	.....	0.71	0.71
Oxygen.....	.....	.....	.....	22.94	.....	.....	.....	22.83
Ash	0.48	.....	.....	.....	0.62	.....	.....	.....
	.....	.....	.....	100.00	.....	.....	.....	100.00

The globulin contained in the "shorts" from the spring wheat was next extracted by treating 2000 grams with 10 per cent sodium-chloride solution for 3 hours, with frequent stirring, and then squeezing out the extract in a screw-press. After the suspended starch had settled, the extract was decanted and saturated with ammonium sulphate. The precipitate was dissolved in 10 per cent sodium-chloride brine, and the solution filtered from the insoluble matter. The clear extract was then dialyzed till free from chlorides, and the precipitated globulin found to consist of well-formed spheroids and masses of confluent spheroids. The globulin was strongly colored, since much coloring matter was extracted from the shorts, rendering the extract a deep red-brown in color. The precipitate was then filtered off, again dissolved in the salt solution and placed in a dialyzer. When free from chlorides, the precipitated globulin was filtered off and found to be still much colored. After partly washing with water it began to dissolve,[1] and was therefore next washed with dilute alcohol and finally with absolute alcohol and with ether. Dried over sulphuric acid, it weighed 2.22 grams, and had the composition shown under the heading "Preparation 25" in the table on page 39.

[1] This solubility of a globulin is due to the formation of acid compounds which are soluble in water, but insoluble in a dilute saline solution. Cf. Osborne, Journal American Chemical Society, 1902, XXIV, p. 39.

The filtrate and washings obtained from this preparation after the second precipitation by dialysis were precipitated by adding a few drops of sodium-chloride solution. The precipitate produced, which was nearly free from coloring matter, weighed 1.25 gram. When analyzed it gave the results shown under the heading "Preparation 26" in the accompanying table.

Preparations 25, 26, and 27.

	Preparation 25.		Preparation 26.		Preparation 27.		
	I.	Ash-free.	I.	Ash-free.	I.	II.	Average.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	50.79	51.00	.....	.....	.....	.....	.....
Hydrogen ...	6.80	6.83	.....	.....	.....	.....	.....
Nitrogen	18.19	18.26	18.56	18.64	18.15	18.43	18.29
Sulphur.....	0.66	0.66	.....	.....	.....	.....	.....
Oxygen.....	.....	23.25	.....	.....	.....	.....	.....
Ash	0.40	.....	0.47	.....	.....	.....	.....
	.....	100.00	.....	.....	.....	.....	.....

The solution filtered from this preparation was next heated to boiling and the coagulum obtained, which weighed 1 gram, when analyzed was found to contain the same amount of nitrogen as the globulin. It was considered to be a coagulated globulin which had been held in solution in water by acid. Its nitrogen content is shown under the heading "Preparation 27."

The total quantity of globulin obtained from the shorts was in this case 4.47 grams, being nearly twice as much as was similarly obtained from a like quantity of the flour.[1]

TABLE 6.—*Summary of analyses of wheat globulin.*

	23.	24.	25.	26.	27.	Average.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	51.07	51.01	51.00	.....	.....	51.03
Hydrogen	6.75	6.97	6.83	.....	.....	6.85
Nitrogen	18.27	18.48	18.26	18.64	18.29	18.39
Sulphur	} 23.91	0.71	0.66	.....	.....	0.69
Oxygen	}	22.83	23.25	.....	.....	23.04
	100.00	100.00	100.00	.....	.....	100.00

[1] Teller (Bull. 42, Ark. Agr. Exp. Sta., 1896) found a much larger quantity of leucosin and globulin nitrogen in the samples of bran which he examined than he found in the flours.

THE PROTEINS OF THE WHEAT EMBRYO SOLUBLE IN SODIUM-CHLORIDE SOLUTION.

The Globulin of the Wheat Embryo.

Wheat-germ meal treated with 10 per cent sodium-chloride brine forms a dense jelly-like mass, from which it is nearly impossible to separate the solution.

With 3 per cent brine a manageable extract can be made by using from six to ten times as much solvent as meal. Thus 100 grams of the meal treated with 600 cc. of 3 per cent sodium chloride yielded in 15 hours 400 cc. of clear filtrate. As has just been shown, the aqueous extract on dialysis, in consequence of a change which affects leucosin, deposits a large amount of protein, chiefly in the coagulated form. In order to obtain preparations of the protein substance soluble in salt solutions, but insoluble in water, which should be free from this coagulable albumin, 2000 grams of germ meal were treated with 20 liters of 3 per cent sodium-chloride solution heated to 70°, whereby the leucosin was coagulated and the salt-soluble globulin brought into solution. The extract, neutral to litmus, was filtered clear, at once saturated with ammonium sulphate, and the proteins thus precipitated collected on a filter, dissolved in water, and the clear solution dialyzed in running water.

Protein matter separated on dialysis in spheroids, which, like many other plant-globulins, united to a plastic mass on the bottom of the dialyzer. This precipitate was dissolved in sodium-chloride solution and, after filtering absolutely clear, dialyzed for 48 hours, the large precipitate which separated allowed to settle, and the solution, which was nearly free from protein, decanted.

A portion of the precipitate was washed first with water, which rendered it opaque and dense, then with dilute and finally absolute alcohol, and dried over sulphuric acid. This weighed 5.22 grams, and is preparation 28. The rest of the precipitate was completely dissolved in 125 cc. of 10 per cent sodium-chloride solution. To this, water was added until its volume was 425 cc., thus making a sodium-chloride solution of nearly 3 per cent. From this diluted solution a gummy deposit separated, from which the fluid was soon completely decanted. The latter was further diluted with 325 cc. of water and the precipitate which resulted allowed to settle to a viscid transparent deposit. From this precipitate the solution was again decanted and dialyzed for 48 hours, but not more than a trace of globulin was deposited. The two precipitates produced by dilution formed preparations 29 and 30, weighing respectively 11.4 grams and 8.15 grams. A part of each of these preparations was set aside for analysis, and the rest, dissolved together in 10 per cent sodium-chloride solution, allowed to stand over night at 4°. The

solution was then decanted from a slight sediment, filtered clear, and heated to 80°, in order to coagulate any leucosin which might be present, and after 2 hours filtered from a very small coagulum which had gradually formed. This filtrate was dialyzed in water for 4 days, and the globulin which separated gave preparation 31. The solution filtered from the first dialysis precipitates, which yielded preparations 28, 29, and 30, was further dialyzed; a little globulin, which separated, was filtered out and the filtrate dialyzed in alcohol for 4 days. A precipitate was produced which, when washed with absolute alcohol and dried, weighed 25 grams. This substance consisted of protein which is described on page 47.

Another series of fractional precipitations of this globulin-like protein was made by extracting 4 kilograms of the oil-free germ meal with 27 liters of 3 per cent sodium-chloride solution heated to 67° at the time it was applied to the meal. The mixture was thoroughly stirred and thrown on filters. A clear filtrate of about 12 liters was finally obtained, which was saturated with ammonium sulphate. The precipitate produced was dissolved in water and its solution dialyzed for 48 hours; whereupon a large quantity of spheroids separated, which on settling united to a coherent mass. This precipitate was washed by decantation with water, dissolved in brine, and its solution made faintly alkaline to litmus by cautiously adding decinormal potassium-hydroxide solution. In order to separate phosphoric acid, a little calcium-chloride solution was then added to this very slightly alkaline liquid, and the latter, though apparently free from any precipitate of calcium phosphate, was filtered, whereby a little suspended matter was removed. The solution was made exactly neutral to litmus by adding 56 cc. decinormal hydrochloric acid and dialyzed for 18 hours. A gummy precipitate (A) adhering to the bottom of the dialyzer then separated, from which the solution (B) was decanted almost completely.

The precipitate (A) was dissolved in about 200 cc. of 5 per cent sodium-chloride solution and the liquid was poured into 800 cc. of water. The resulting flocculent precipitate settled rapidly to a coherent deposit, from which the solution was decanted. The deposit was repeatedly washed by decantation with water, which caused it to lose its gummy character and become opaque, white, and granular. It weighed 15.5 grams and was preparation 32. The solution marked B was further dialyzed for 48 hours, when a second precipitate formed, which, like 32, completely dissolved in sodium-chloride solution to a solution perfectly neutral to litmus. This precipitate was washed by decantation with water, but the finer part settled so slowly that it was necessary to decant it together with the water. The sediment, after exhausting with absolute alcohol and drying, weighed 23.5 grams, and formed preparation 33. On long standing the decanted washings deposited the finely divided matter, which was then collected on a

filter, dissolved in sodium-chloride solution, and precipitated by water, giving 15.4 grams of preparation 34.

To determine the quantity of globulin contained in the oil-free germ meal, 200 grams of the meal were treated with 2000 cc. of 3 per cent sodium-chloride solution heated to 65° and the extract filtered perfectly clear. Of this, 1000 cc. were dialyzed until free from chlorides, when the precipitate of spheroids was filtered out. This preparation, 35, formed 5.05 per cent of the oil-free meal.

To obtain a quantity of this globulin for digestion with pepsin, a quantity of germ meal was extracted with 3 per cent sodium-chloride solution heated to 70°; the extract was filtered clear and saturated with ammonium sulphate. The precipitate produced was dissolved in water and the resulting gummy and somewhat turbid solution filtered clear. The filtrate was dialyzed until the solution gave no turbidity on pouring into distilled water. The protein, which had then separated in spheroids, weighed 27.3 grams, preparation 36.

A part of the extract from which 36 had been prepared was mixed with an equal volume of decinormal potassium hydroxide solution—about twice the quantity necessary to neutralize the extract to phenolphthalein. The solution was then dialyzed in distilled water frequently renewed, and in this way a considerable quantity of phosphorus was separated in the alkaline dialysate. When all, or nearly all, which it was possible to separate in this way had been removed, the solution in the dialyzer was neutralized with hydrochloric acid until it no longer reacted alkaline to litmus. This caused a turbidity. The acid was then further added until an acid reaction with litmus was obtained, producing a precipitate from which, after settling, the solution was decanted. The precipitate was then dissolved in sodium-chloride solution, its solution filtered clear and dialyzed, whereby a substance was precipitated in spheroids, which formed preparation 37, weighing 3.0 grams. These preparations had the composition shown in table 7.

TABLE 7.—*Composition of preparations extracted by sodium-chloride solution from the wheat embryo.*

	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	.....	.....	48.77	50.03	50.23	48.17	49.39	48.75	49.79	48.67
Hydrogen	.....	.....	6.44	7.04	6.89	6.54	6.78	6.52	6.76	6.56
Nitrogen	18.14	18.21	18.12	18.39	18.23	18.06	17.95	18.16	18.01	17.97
Sulphur	0.49	0.56	0.51	0.60	0.53	0.55	0.48	0.63	0.61	0.61
Phosphorus	1.15	1.03	1.35	0.76	0.56	1.41	1.17	1.41	1.11	1.55
Ash	2.29	1.86	2.25	1.30	1.22	3.85	2.60	2.66	1.11	2.94
P_2O_5 in ash	1.66	1.34	1.68	0.84	0.80	2.00	1.82	2.00	0.68	2.30

These analyses, when calculated free from nucleic acid and ash, as was done for the albumin preparations, in the manner described on page 27, gave the results set out in table 8.

TABLE 8.—*Composition of the globulin contained in the preparations extracted from the wheat embryo by sodium-chloride solution.*

	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	.....	.....	51.37	51.58	51.40	51.56	51.86	51.40	51.98	51.70
Hydrogen..	.....	.....	6.83	7.31	7.08	7.07	7.19	6.94	7.12	7.05
Nitrogen...	18.59	18.59	18.62	18.70	18.45	18.85	18.41	18.71	18.37	18.53
Sulphur	0.57	0.63	0.60	0.66	0.57	0.67	0.55	0.75	0.70	0.75
Oxygen....	.....	.....	22.58	21.75	22.50	21 85	21.99	22.20	21.83	21.97
	.....	.....	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

These figures plainly indicate that these globulin preparations are mixtures of nucleates of protein substance of the same ultimate composition, and contain from 5 to 15 per cent of nucleic acid. This protein has very nearly the same composition as the globulin occurring in the kernel of wheat, rye, barley, and maize. In the entire kernel so little of this globulin is present that it is difficult to prepare it pure therefrom. For this reason the analyses given in table 9 do not agree as closely as they might otherwise be expected to. From the whole seed this globulin is obtained entirely free from phosphorus, which is probably due to the much greater proportion of protein matter to nucleic acid in the entire seed compared with that existing in the embryo.

TABLE 9.—*Composition of the globulin contained in various cereals.*

	Wheat embryo.	Wheat kernel.	Rye[1] kernel.	Maize[2] kernel.	Barley[3] kernel.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	51.57	51.03	51.19	51.99	50 88
Hydrogen.....	7.07	6.85	6.74	6.81	6.65
Nitrogen.....	18.60	18.39	18.19	18.02	18.10
Sulphur	0.65	0.65	} 23.88	{ 0.66	} 24.37
Oxygen.....	22.11	23.08		{ 22.52	
	100.00	100.00	100.00	100.00	100.00

[1] Osborne, Journal American Chemical Society, 1895, XVII, p. 429.

[2] Chittenden & Osborne, American Chemical Journal, 1891, XIII, pp. 327, 385, and 1892, XIV, p. 20.

[3] Osborne, Journal American Chemical Society, 1895, XVII, p. 539.

Having determined the composition of this globulin-like protein and also that of the albumin, it became clear that several preparations obtained from the aqueous extract were mixtures of these two substances, thus showing the globulin to be present to some extent in the aqueous extract.

As noted on page 24, when 2000 cc. of an aqueous extract of about 650 grams of the meal were dialyzed in running water for 4 days a dense turbidity was formed, which could not be removed by filtration. This, however, on adding a little hydrochloric acid, was converted into a precipitate, which was readily dissolved by adding sodium chloride sufficient to make a 3 per cent solution, and was precipitated from this solution by dialysis.

Preparation 38, weighing 9 grams, was thus obtained, which, dried at 110°, had a composition which corresponds pretty nearly with that of a mixture of 60 per cent of the globulin with 40 per cent of leucosin, except that the amount of sulphur found was somewhat greater than that calculated. The analysis is shown in the following table:

Preparations 38 and 39.

	Preparation 38.					Preparation 39.		
	I.	II.	Average.	Corrected for ash and nucleic acid.	Calculated for 60 per cent globulin and 40 per cent leucosin.	I.	Corrected for ash and nucleic acid.	Calculated for 40 per cent globulin and 60 per cent leucosin.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	48.30	47.92	48.11	51.70	51.95	49.49	51.80	52.13
Hydrogen	6.49	6.41	6.45	7.07	7.07	6.81	7.14	7.03
Nitrogen	17.40	17.24	17.32	17.74	17.74	16.87	17.32	17.30
Sulphur	0.83	0.85	0.84	1.08	0.91	0.93	1.14	1.05
Phosphorus	1.91	.....	1.91	.....	.....	0.89	.....	.....
Oxygen	.....	.....	.....	22.51	22.53	.....	22.60	22.49
Ash	3.95	.....	.....	.....	.....	4.00	.....	.....
P_2O_5 in ash	2.95	.....	.....	.....	.....	2.01	.....	.....
	.....	.....	.....	100.00	100.00	.....	100.00	100.00

After heating another portion of the same aqueous extract to 65° for some time and filtering off the coagulum, the filtrate was dialyzed for 5 days into alcohol and the precipitate thereby produced filtered out and exhausted with water. The residue of protein matter coagulated by alcohol, weighing 6.7 grams and marked preparation 39, was then dried at 110° and analyzed with the results shown in the preceding table.

This analysis corresponds quite nearly with that of a mixture of 40 per cent of the globulin with 60 per cent of leucosin.

No earlier analyses of the wheat globulin are on record. Weyl[1] was the first to call attention to the presence of globulin in wheat, and says that "besides vegetable-vitellin I detected in the 10 per cent sodium-chloride extract of the pulverized seeds of wheat, peas, oats, white mustard, and sweet almonds a second globulin substance." This he calls vegetable-myosin and gives its coagulation-point at 55° to 60°. It is probable that Weyl in some way mistook for a globulin the albumin already described.

Later, Weyl & Bischoff[2] state:

On investigating the proteins of wheat, one of us found chiefly an albuminous substance which, on account of its resemblance to myosin, was named vegetable-myosin. This vegetable-myosin must be the mother-substance of the gluten, since in wheat meal, together with it, other nitrogenous substances exist, at the most, only in very small amount.

On what experimental evidence this statement rests the writer has been unable to discover, and in view of his experience he is at a loss to understand it.

Martin[3] considers wheat flour to contain a large amount of globulin of the myosin type, coagulating between 55° and 60°, precipitated by saturation with sodium chloride and ammonium sulphate. Here again the small quantity of albumin contained in the flour has evidently been mistaken for a large quantity of vegetable-myosin. This perhaps is not surprising, as the precipitates obtained by saturating sodium-chloride extracts with ammonium sulphate appear very bulky, and in the absence of an actual determination of the weight of these precipitates misleading conclusions might easily be reached. The only globulin found by the writer in extracts of wheat meal, either winter or spring wheat, is the one just described, which in properties and composition closely resembles those globulins which have been found in other seeds.

THE PROTEOSE OF WHEAT FLOUR.

As already stated in describing the reaction of the extract freed from globulin by dialysis, there was found in it one or more proteoses, besides the albumin just described. These were almost wholly precipitated by saturation with sodium chloride or by adding 20 per cent of this salt to the solution, together with a little acetic acid.

If the albumin is completely removed by heat and the filtered solution then concentrated, a coagulum gradually develops. This substance must be derived from the proteose-like protein, as this forms nearly if not quite all the protein substance remaining in solution before concentration. If

[1] Weyl, Zeitschrift für physiologische Chemie, 1877, I, p. 72.
[2] Weyl & Bischoff, Berichte der deutschen chemischen Gesellschaft, 1880, XIII, p. 367.
[3] Martin, British Medical Journal, 1886, II, p. 104.

the coagulum is removed by concentration and long-continued heating and subsequent filtration, wholly uncoagulable proteose-like substances are found in solution. The amount of proteose is extremely small, and no preparations were made for analysis. The coagula obtained by concentrating the solution filtered from preparations 3 and 4, respectively, were analyzed with the results shown in the accompanying table under the head "Preparation 40."

Another coagulum similarly obtained from the solution filtered from preparation 4 was also analyzed and the following figures obtained:

Preparations 40 and 41.

	Preparation 40.				Preparation 41.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	51.35	51.60	51.48	51.62	51.73	.....	51.73	51.86
Hydrogen...	.....	.....	.....	.....	6.82	.....	6.82	6.82
Nitrogen....	16.79	16.58	16.69	16.73	17.29	17.26	17.28	17.32
Sulphur.....	.....	.....	.....	.....	}	.....	.....	24.00
Oxygen.....	.....	.....	.....	.....				
Ash.....	0.27	.....	.....	.....	0.25	.....	0.25	.....
	.....	.....	.....	.....	.....	.....	.....	100.00

THE PROTEOSE OF THE WHEAT EMBRYO.

In making the preparations from the embryo already described considerable quantities of crude proteose were obtained from both the aqueous and sodium-chloride extracts. After the leucosin and the globulin had been separated as completely as possible, the solutions containing the proteoses were dialyzed in alcohol, and the precipitates produced were washed and dried over sulphuric acid.

A mixture weighing 15.4 grams was made by uniting several such preparations that had been obtained from aqueous extracts from which most of the other proteins had been separated, without heat, by saturating with sodium chloride and dialysis in alcohol. The mixture contained much matter made insoluble in water by the final treatment with alcohol. This was filtered out, washed thoroughly with water and with alcohol, and when dried weighed 4.18 grams, and was marked preparation 42. The filtrate from this was saturated with ammonium sulphate, the precipitate redissolved, and again precipitated in the same way. The solution of the second precipitate was dialyzed in cold distilled water until free from sulphate, and then for several days in alcohol; the precipitate thus produced was dissolved in water, a little insoluble matter filtered out, and its clear solution saturated

with sodium chloride, which produced a small precipitate. This was filtered out, dissolved, and its solution dialyzed in water. The salt-saturated filtrate was likewise dialyzed, and when both solutions were free from chlorine the dialyzers were transferred to alcohol and the proteose thereby precipitated. The proteose separating on saturation with salt gave 0.6 gram of preparation 43; that from the salt-saturated solution 0.97 gram of preparation 44. This small yield of proteose indicates that the greater part had diffused through the parchment paper during the long dialysis to which the solutions had been subjected.

Another crude product was obtained by dialyzing an aqueous extract in alcohol after separating the leucosin which had been coagulated by heat. This, weighing 35 grams, was dissolved in water and the insoluble matter filtered out, washed and dried, giving preparation 45, weighing 7.26 grams.

The filtered solution was saturated with ammonium sulphate, the precipitate dissolved in water, and the clear solution dialyzed in distilled water until free from sulphates, and then in alcohol. The substance thus separated was again dissolved in water and its solution saturated with salt; the precipitate thus produced was dissolved in water, and its solution, as well as the salt-saturated filtrate, were dialyzed in water. When free from chlorine, these solutions were dialyzed in alcohol and yielded, respectively, preparations 46, weighing 4 grams, and 47, weighing 1.84 grams.

Another preparation of crude proteose was obtained by extracting the meal as described on page 41 with 3 per cent sodium-chloride solution heated to 70°, dialyzing the extract in water, coagulating the leucosin by heat, and precipitating the proteose by dialysis in alcohol. A mixture of such preparations, weighing 31.6 grams, was treated with water, the insoluble matter filtered out, washed, and dried, giving 5.16 grams of preparation 48.

The filtered solution was saturated with ammonium sulphate, the precipitate dissolved in water, the solution dialyzed in distilled water till free from sulphate, and then in alcohol. The separated proteose was redissolved in water and its solution saturated with sodium chloride. The precipitate which resulted was filtered out, dissolved in water, and its solution, as well as the salt-saturated filtrate, were dialyzed in water till free from chlorine, and finally in alcohol.

The products thus obtained formed, respectively, preparation 49, weighing 0.75 gram, and 50, weighing 1.35 grams. One other proteose preparation was made from the aqueous extract described on page 26, from which the phosphorus was largely separated by making it slightly alkaline and adding calcium chloride. After heating the extract to boiling and filtering out the coagulum, the filtrate was dialyzed in alcohol, the resulting precipitate dehydrated with absolute alcohol, dried over sulphuric acid, redissolved in water, and precipitated by saturating with ammonium sulphate.

The gummy precipitate, having the general appearance and properties of similar precipitates of the proteoses obtained by the action of pepsin, was dissolved in water, dialyzed free from sulphates, and then precipitated by dialysis in alcohol, giving 2 grams of preparation 51. These preparations were dried at 110° and analyzed with the results shown in table 10.

TABLE 10.—*Composition of alcohol coagula and of proteose prepared from the wheat embryo.*

	Residues of other proteins coagulated by alcohol.			Proteose precipitated by sodium chloride.			Proteose soluble in saturated NaCl solution.			
	42.	45.	48.	43.	46.	49.	44.	47.	50.	51.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.36	49.44	51.93	.....	49.94	.....	48.46	48.70	48.44	48.99
Hydrogen	6.98	6.85	6.87	.....	6.80	.....	6.70	6.73	6.71	6.85
Nitrogen	16.01	16.00	16.30	16.79	17.08	16.26	16.91	16.76	16.16	16.89
Sulphur	1.85	4.08	1.30	.....	1.24	.....	} 27.93	27.81	28.69	{ 1.10
Oxygen	22.80	23.63	23.60	.....	24.94	.....	}			{ 26.17
	100.00	100.00	100.00	.....	100.00	.....	100.00	100.00	100.00	100.00
Ash	0.81	14.13	0.95	.....	0.30	0.77	1.13	1.00	0.74	1.27

From these analyses it is seen that the matter insoluble in water, forming preparations 42, 45, and 48, consists of coagulated protein apparently mostly derived from leucosin. The high proportion of sulphur in 42 and 45 is due to calcium sulphate precipitated by alcohol from the aqueous extract. The remaining preparations have the low percentage of carbon characteristic of proteoses made by pepsin digestion.

THE PROPORTIONS OF THE VARIOUS PROTEIN SUBSTANCES OF THE WHEAT EMBRYO.

Twenty grams of fresh germ meal, from which the ether-soluble constituents had *not* been separated, were treated with 500 cc. of water, and after shaking for some time the extract was filtered clear. Two portions of 100 cc. each were treated with a few drops of very dilute hydrochloric acid and heated in a boiling water-bath. The coagulum which separated was collected on a filter and its nitrogen determined. To the filtrate from one coagulum tannin was added, and nitrogen was determined both in the precipitate and in the filtrate. Another lot of 20 grams was treated in the same way and nitrogen determined in the heat-coagulum formed in each of two portions of 100 cc. The amount of nitrogen corresponding to 1 gram of germ meal was found in the four coagula to be 0.0163 gram, 0.0156 gram, 0.0159 gram, and 0.0162 gram, in the tannin precipitate 0.0062 gram, and in the solution filtered from the latter 0.0062 gram.

Twenty grams of germ meal were extracted with 500 cc. of 3 per cent sodium-chloride solution heated to 70°, whereby the leucosin was coagulated and the globulin and proteose dissolved. Of the clear filtered extract 100 cc. yielded with tannin a precipitate containing 0.0166 gram nitrogen per gram of meal extracted.

Two portions of the meal, each of 1 gram, were exhausted with 3 per cent sodium-chloride solution heated to 70° and nitrogen determined in the residues. The 0.0331 gram and 0.0309 gram of nitrogen found in the residues were from the leucosin and insoluble nitrogenous bodies, so that the nitrogen belonging to the latter equaled 0.0171 gram and 0.0149 gram. From the average of these figures we find the following amounts of the different forms of nitrogen in 1 gram of the wheat-germ meal:

	N, grams.
Insoluble in water and salt solution	.0160
Insoluble in water, but soluble in salt solution (globulin nitrogen)	.0100
Soluble in water and coagulable by heat (albumin nitrogen)	.0160
Soluble in water, uncoagulable by heat, precipitable by tannin (proteose nitrogen)	.0050
Not precipitable by tannin (non-protein nitrogen)	.0060
Total	.0530
Found by direct nitrogen determination	.0531

It has been shown that the coagulated leucosin preparations contain about 10 per cent of nucleic acid, the globulin about 15 per cent, while those of the proteose contain none. Deducting these quantities from the nitrogen given above, it is found that 9.5 per cent of the embryo is leucosin, 4.84 per cent globulin, and 3.03 per cent proteose.

The bodies which are represented by the insoluble nitrogen could not be separated from the embryo. The residue, after extraction with hot-salt solution, contained 0.0076 gram of phosphorus. In view of the large proportion of nucleic acid found in the extracts of the embryo, it is not improbable that this phosphorus mostly belongs to nucleic acid, and that the insoluble nitrogen largely belongs to compounds of protein with relatively much nucleic acid.

DIGESTION OF THE PHOSPHORUS-CONTAINING PROTEIN PREPARATIONS WITH PEPSIN-HYDROCHLORIC ACID.

Leucosin Nucleate.

Ten grams of the coagulated albumin, preparation 8, were suspended in 400 cc. of water and dissolved by adding 100 cc. of decinormal potassium-hydroxide solution. To the nearly clear solution which resulted an equal volume of 0.4 per cent hydrochloric acid was added, together with some pepsin, and the mixture digested at 37°. In a short time the solution

became perfectly clear, but later deposited a large coherent precipitate, which gradually contracted, but at the same time retained the form of the lower part of the beaker. From this the clear solution was decanted, the precipitate thoroughly washed by decantation, suspended in water and dissolved by adding 28 cc. of decinormal potassium-hydroxide solution, an amount of alkali just sufficient to dissolve all the substance, and at the same time make the solution neutral to litmus. When to this solution decinormal acid was gradually added, no precipitate appeared until nearly one-half the quantity of acid required for complete neutralization had been added, but with 28 cc. the solution was neutralized and the nuclein completely precipitated, the addition of 2 cc. more acid giving no turbidity in the filtered solution. This precipitate formed preparation 52, weighing 1.54 grams.

To precipitate this substance a quantity of acid was added exceeding that of the alkali employed for solution by just 2 cc. The filtrate from the precipitate, however, required not 2 cc. of alkali, but 8.5 cc. for neutralization to phenolphthalein, showing 6.5 cc. of alkali to have been neutralized by the acid of the nuclein originally dissolved. The neutralized filtrate left on evaporation 0.3975 gram of substance, the aqueous solution of which was precipitated by hydrochloric or nitric acid, but *not* by ammonium molybdate solution until after boiling with acid for some little time, when yellow phosphomolybdate was precipitated. These facts indicate the presence in this filtrate of a nucleic acid.

More nuclein was made from the same preparation, 7, by suspending 30 grams in 0.2 per cent hydrochloric acid containing pepsin, which, even at 20°, caused within 2 hours complete solution of the coagulated protein. The solution was digested at 37° for 48 hours, during which time much nuclein separated, having the appearance and properties of the preparation just described.

After decanting the clear solution and thoroughly washing the residual nuclein, the latter was suspended in water and dissolved in 72 cc. decinormal potassium-hydroxide solution. The solution thus obtained was made neutral to litmus by adding 11 cc. of decinormal hydrochloric acid, but no precipitate appeared till 1.5 cc more of acid were added. To the solution 72 cc. decinormal hydrochloric acid were added, giving a precipitate, preparation 53, which weighed 3.4 grams. The filtrate from this precipitate, as in the former case, was strongly acid, requiring 12 cc. of decinormal potassium hydroxide to neutralize it to phenolphthalein. Two other preparations of nuclein were made from 8.493 grams of 13 and 9.804 grams of 16, both being substances precipitated from the aqueous extract by saturating with sodium chloride. Each portion was suspended in about 300 cc. of 0.2 per cent hydrochloric acid, containing 0.1 gram of pepsin, and, with frequent

stirring, digested at 40° for 24 hours. Throughout the digestion a large part of the substance remained undissolved. An equal volume of 0.2 per cent hydrochloric acid, containing 0.1 gram of pepsin, was again added to each, and the digestion continued for 24 hours longer. The insoluble matter which remained was not coherent like the two former nuclein products, but consisted of a white, very finely divided substance, which was easily filtered out and washed. From 13, 4.04 grams of preparation 54 were obtained, and from 16, 4.16 grams of 55.

Globulin Nucleate.

Fifteen grams of a mixture of nearly equal parts of the globulin preparations 32 and 33 were next suspended in 0.2 per cent hydrochloric acid containing 0.2 gram of pepsin, which, within a short time, almost completely dissolved the protein matter. From this solution, on further digestion, the nuclein separated, forming a coherent deposit. After 72 hours' digestion the clear solution was decanted, the deposit dissolved in a little ammonia, and its solution filtered perfectly clear from a very slight gelatinous residue. The solution was then treated with acetic acid added in excess of the amount necessary to neutralize it to litmus. Since, even on standing, the precipitate so produced separated imperfectly, an equal volume of alcohol was added. The substance, which then separated well, gave 2.38 grams of preparation 56, or about 16 per cent of the original substance.

The filtrate from the acetic acid precipitate, on adding hydrochloric acid, gave a further slight precipitate, which had properties characteristic of nucleic acid.

Still another preparation of nuclein was made from the globulin by suspending 10 grams of 36 in water and adding 50 cc. of decinormal potassium-hydroxide solution. This solution was neutralized and an equal volume of 0.4 per cent hydrochloric acid at once added, producing a turbid solution, which, however, contained no visible particles. To this pepsin was added and the mixture digested for 40 hours, during which time a coherent deposit of nuclein formed on the bottom of the beaker. From this the clear solution was decanted. The deposit was then thoroughly washed with water and dissolved in 43 cc. of decinormal potassium-hydroxide solution. To this clear solution 43 cc. of decinormal hydrochloric acid were added, causing a gummy precipitate, which could not be filtered until 15 cc. more acid had been added, when the precipitate rapidly settled as a coherent deposit, from which the solution was soon decanted. This solution required for neutralization to litmus 16 cc. of decinormal potassium-hydroxide solution, and to phenolphthalein 18 cc. The precipitate when washed and dried gave 2.2 grams of preparation 57.

These six preparations were all dried at 110° and analyzed with the results shown in table 11.

TABLE 11.—*Composition of nuclein from the proteins of the wheat embryo.*

	52.	53.	54.	55.	56.	57.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	44.87	44.35	42.68	43.35	39.42	41.92
Hydrogen.....	5.82	5.77	5.45	5.47	5.03	5.25
Nitrogen.....	16.04	16.64	16.12	16.01	16.05	17.00
Sulphur.....	0.97	1.03	0.65	0.85	0.53	0.46
Phosphorus...	4.58	5.07	5.32	4.88	5.27	5.63
Ash	0.60	0.78	1.72	1.72	17.42	1.17
P_2O_5 in ash....	0.29	0.55	1.24	0.94	10.56	0.69
Bases in ash...	0.31	0.23	0.48	0.78	6.86	0.48

These analyses of nuclein were calculated free from the bases of the ash and from nucleic acid, in the way previously described, with the results given in table 12.

TABLE 12.—*Composition of protein matter contained in the nuclein.*

	52.	53.	54.	55.	56.	57.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	53.65	54.77	51.80	52.36	52.30	51.64
Hydrogen.....	7.23	7.46	6.85	6.73	6.91	6.60
Nitrogen.....	16.68	17.56	16.31	16.31	19.31	18.93
Sulphur.....	1.98	2.37	1.61	1.89	1.53	1.25
Oxygen.....	20.46	17.84	23.43	22.71	19.95	21.58
	100.00	100.00	100.00	100.00	100.00	100.00

The composition of the protein matter in 54 and 55 is very nearly that of leucosin, except as regards sulphur, the amount of which is decidedly greater. On the other hand, 52 and 53, which also were derived from preparations whose protein matter was leucosin, differ in composition very decidedly from that substance. This is probably because on pepsin digestion the substance of preparations 54 and 55 remained throughout undissolved, whereas 52 and 53 separated on pepsin digestion from nearly clear solutions, and therefore doubtless their protein matter had been to some degree altered by the pepsin before separating as an insoluble compound with nucleic acid. The two nucleins, 56 and 57, from the globulin, which also had separated from solution, show similar differences in composition when compared with the unaltered globulin, carbon and nitrogen being higher and sulphur very much higher than in the globulin.

PROTEIN SOLUBLE IN DILUTE ALCOHOL—GLIADIN.

As already stated, wheat flour yields to dilute alcohol a considerable amount of protein matter. Treatment of the residue remaining after extracting the flour with 10 per cent sodium-chloride brine likewise removes a large amount of protein, as does also extraction of the gluten obtained by washing the dough with water. Extracts were made with alcohol under all these conditions, and the protein extracted subjected to repeated fractional precipitations.

Direct Extraction with Dilute Alcohol.

5000 grams of the straight flour were extracted with 10 liters of alcohol, 0.90 sp. gr., and allowed to soak over night. The next morning the mixture was stirred, and, after settling, the clear solution poured off. Three liters more of alcohol, 0.90 sp. gr., were then added, and after standing some time the supernatant liquid was decanted and the residue squeezed nearly dry. The solution so obtained was designated "extract 1." The residue was again treated with 4 liters of 0.90 sp. gr. alcohol and pressed nearly dry. This formed extract 2. The same process twice repeated gave two extracts which, when united, formed extract 3. Each of these three extracts, after filtering perfectly clear, was separately concentrated to one-third its volume, and, after cooling, decanted from the very glutinous, viscid mass which had separated. 1 and 3 yielded much more substance than 2. On stirring with a glass rod the precipitated mass formed a very thick, viscid liquid. This substance was in each case dissolved in a small amount of hot alcohol of 0.90 sp. gr., in which it was very soluble, and the solution was allowed to cool over night. Most of the substance separated on cooling and the liquid was decanted from it. The solution decanted from the second and third extracts was treated with a quantity of distilled water and a little sodium chloride added. This threw down a small precipitate, which on standing collected on the bottom of the vessel as a clear semi-fluid mass. This was treated with water, absolute alcohol, and ether, and yielded 7.27 grams of preparation 58 from extract 2, and 10.7 grams of the preparation 59 from extract 3. These had the following compositions :

Preparations 58 and 59.

	Preparation 58.		Preparation 59.	
	I.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*
Nitrogen	17.05	17.18	17.15	17.26
Ash	0.76	.....	0.65	.....

On examination both were found to contain some fat which could not be wholly removed, as the substance had dried in a dense, horny form. The residues which had separated from the solutions just described were next washed by thoroughly intermixing with distilled water. The water was found to dissolve some of the protein, which was subsequently precipitated by the addition of a little sodium-chloride solution. After standing over night this precipitate settled to the bottom of the vessel in a transparent layer, from which the solution could be completely decanted. After treating this substance with absolute alcohol, it formed a voluminous white, porous mass, which was digested for some time with ether. From extract 1 preparation 60 was obtained, weighing 12.4 grams; from extract 2, united with that from 3, preparation 61, weighing 8.6 grams.

These preparations were found on analysis to have the following compositions:

Preparations 60 and 61.

	Preparation 60.				Preparation 61.	
	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	52.40	52.58	52.49	52.52	52.69	52.77
Hydrogen ..	6.77	6.78	6.78	6.78	6.77	6.78
Nitrogen ...	17.52	17.73	17.63	17.64	17.74	17.77
Sulphur....	1.05	1.11	1.08	1.08	1.26	1.26
Oxygen	.....	.....	.....	21.98	.....	21.42
Ash.....	0.06	.....	.....	.....	0.15	.....
	.....	.....	.....	100.00	.....	100.00

The residues remaining after washing with distilled water were then digested with alcohol of 0.820 sp. gr., which dissolved much of the substance. After standing some time, the strong alcoholic solutions were decanted from the residues and found to consist of milky liquids. The addition of a few drops of 10 per cent sodium-chloride solution immediately produced in each a very large, curdy precipitate, the liquids from which they separated being left perfectly clear. From the solution from the first extract, which was about one liter in volume, 32.26 grams of substance were obtained after dehydration with absolute alcohol and digestion with ether. This was marked preparation 62. From the second extract was similarly obtained preparation 63, weighing 5.34 grams, and from the third extract preparation 64, weighing 17.43 grams. The filtrates from 62 and 63 were found to be almost wholly free from protein, but that from 64 left on evaporation a residue which, when freed from fat, weighed 7.53 grams, preparation 65.

These preparations were found to have the following compositions:

Preparations 62 and 63.

	Preparation 62.		Preparation 63.			
	I.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	52.59	52.67	52.28	52.54	52.41	52.55
Hydrogen..	6.70	6.71	6.87	6.79	6.83	6.85
Nitrogen...	17.64	17.66	17.90	.....	17.90	17.94
Sulphur.....	1.22	1.22	1.21	.....	1.21	1.21
Oxygen	.....	21.74	.....	.....	.....	21.45
Ash	0.15	.....	0.27	.....	.....	.....
	.....	100.00	.....	.....	.....	100.00

Preparations 64 and 65.

	Preparation 64.				Preparation 65.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	52.52	52.82	52.67	52.74	52.13	52.42	52.28	52.39
Hydrogen..	6.72	6.79	6.76	6.77	6.97	6.85	6.91	6.93
Nitrogen...	17.60	.....	17.60	17.62	17.35	17.19	17.27	17.31
Sulphur....	1.23	.....	1.23	1.23	1.35	1.41	1.38	1.38
Oxygen	.....	.....	.....	21.64	.....	.....	.....	21.99
Ash	0.14	.....	.....	.....	0.22	.....	.....	.....
	.....	.....	.....	100.00	.....	.....	.....	100.00

The residues which remained after treatment with alcohol of 0.820 sp. gr. were then dehydrated with absolute alcohol and digested with ether. From extract 1 preparation 66 was obtained, weighing 63 grams; from extract 2 preparation 67, weighing 2.1 grams, and from extract 3 preparation 68, weighing 41.2 grams.

Preparation 66, which constituted the principal fraction of the protein extracted, was further treated in the following manner: 20 grams were dissolved in 250 cc. of 0.90 sp. gr. alcohol and found to yield a clear solution, which was then poured into 800 cc. of absolute alcohol, whereby a considerable precipitate was at once separated, leaving the solution milky. This substance was dehydrated with absolute alcohol and digested with ether, yielding preparation 69. The filtrate was then treated with a few drops of 10 per cent sodium-chloride solution, causing a heavy precipitate, which on stirring rapidly agglutinated and adhered as a mass to the stirring-

rod. This was removed, treated in the usual manner, and marked preparation 70. The mother-liquor from which this separated, after standing over night, deposited a further small amount of protein, which, after treatment with absolute alcohol and ether, gave preparation 71.

These six substances were analyzed and found to have the following compositions :

Preparations 66 to 71.

	Preparation 66.		Preparation 67.			
	I.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.81	52.84	.....	.....	.....	.....
Hydrogen	6.81	6.81	.....	.....	.....	.....
Nitrogen	17.66	17.67	15.42	15.28	15.35	15.42
Sulphur	1.11	1.11	.....	.....	.....	.....
Oxygen	.....	21.57	.....	.....	.....	.....
Ash	0.06	.....	0.42	.....	0.42	.....
	.....	100.00	.....	.....	.....	.....

	Preparation 68.				Preparation 69.	
	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.90	52.99	52.95	53.02	.....	.....
Hydrogen	6.74	6.82	6.78	6.79	.....	.....
Nitrogen	17.24	17.36	17.30	17.32	17.67	17.69
Sulphur	1.05	.....	1.05	1.05	.....	.....
Oxygen	.....	.....	.....	21.82	.....	.....
Ash	0.15	.....	.....	.....	0.10	.....
	.....	.....	.....	100.00	.....	.....

	Preparation 70.				Preparation 71.	
	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.15	52.35	52.25	52.33	52.28	52.38
Hydrogen	6.93	6.87	6.90	6.91	7.12	7.13
Nitrogen	17.52	17.84	17.68	17.70	17.79	17.82
Sulphur, Oxygen	.....	.....	.....	23.06	.....	22.67
Ash	0.15	.....	.....	.....	0.19	.....
	.....	.....	.....	100.00	.....	100.00

If the preceding analyses are brought together as in table 13, the effect of the various fractional solutions and precipitations may be seen at a glance.

TABLE 13.—*Gliadin extracted by direct treatment of the flour with alcohol.*

	From 0.9 sp. gr. alcoholic solution.		From water washings.		From 0.82 sp. gr. alcoholic solution.		
	58.	59.	60.	61.	62.	63.	64.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	.....	.....	52.52	52.77	52.67	52.55	52.74
Hydrogen	.....	.....	6.78	6.78	6.70	6.85	6.77
Nitrogen	17.18	17.26	17.64	17.77	17.66	17.94	17.62
Sulphur	.....	.....	1.08	1.26	1.22	1.21	1.23
Oxygen	.....	.....	21.98	21.42	21.75	21.45	21.64
	.....	.....	100.00	100.00	100.00	100.00	100.00
Weight of substance in grams.	7.27	10.70	12.40	8.60	32.26	5.34	17.43

	From filtrate from 64.	Residue after extraction with 0.820 sp. gr. alcohol.			Fractional reprecipitations of preparation 66.		
	65.	66.	67.	68.	69.	70.	71.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.39	52.82	.....	53.02	.....	52.33	52.38
Hydrogen	6.93	6.81	.....	6.79	.....	6.91	7.13
Nitrogen	17.31	17.67	15.42	17.32	17.69	17.70	17.82
Sulphur	1.38	1.11	.....	1.05	.....	23.06	22.67
Oxygen	21.99	21.57	.....	21.82	.....		
	100.00	100.00	.....	100.00	.....	100.00	100.00
Weight of substance in grams.	7.53	63.0	2.10	41.20	.....	.....	.....

It is evident that 58 and 59 contain less nitrogen than the great bulk of the protein extracted. This is due to fat which they were found to contain and which could not be wholly removed by extraction with ether, since in drying these preparations were converted into the horny condition which rendered penetration with ether impossible.

Preparation 67 was evidently impure, as might be expected, since it contained all of the insoluble particles of the entire extract which had escaped filtration, and owing to its small amount these impurities produced a marked effect on its percentage composition. The same is true of 68, but as the quantity of this preparation is so much greater this contamination has produced much less effect on its composition. Preparation 65 was obtained by evaporating the mother-liquor from 64 nearly to dryness and then extracting with absolute alcohol and ether. It would hardly be expected that under

such circumstances it would be entirely pure. The analyses of the other preparations are in good agreement, and it is evident that no fractional separation of the extracted protein is indicated. The preparations obtained from solution in pure water have the same composition as those from solutions in alcohol of 0.820 sp. gr., and also the same composition as the residue remaining after treatment with each of these reagents.

The total amount of protein contained in these several preparations is 207.83 grams, being equal to 4.16 per cent of the flour.

TABLE 14.—*Gliadin extracted from flour with dilute alcohol after extraction with 10 per cent sodium-chloride solution.*

	Preparation 72.				Preparation 73.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.61	.....	52.61	52.69	52.65	.....	52.65	52.72
Hydrogen	6.82	.....	6.82	6.84	6.85	.....	6.85	6.86
Nitrogen	17.62	17.78	17.70	17.73	17.87	.....	17.87	17.89
Sulphur	1.00	1.04	1.02	1.02	0.95	0.94	0.95	0.95
Oxygen	.....	.....	.....	21.72	.....	.....	.....	21.58
Ash	0.16	.....	.....	.....	0.13	.....	.....	.....
	.....	.....	.....	100.00	.....	.....	.....	100.00

	Preparation 74.				Preparation 75.		Preparation 76.	
	I.	II.	Average.	Ash-free.	I.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.52	52.67	52.60	52.71	.....	.....	52.62	52.65
Hydrogen	6.82	6.76	6.79	6.81	.....	.....	6.83	6.83
Nitrogen	17.72	.....	17.72	17.75	16.93	17.08	17.78	17.79
Sulphur	1.10	.....	1.10	1.10	.....	.....	1.08	1.08
Oxygen	.....	.....	.....	21.63	.....	.....	.....	21.65
Ash	0.21	.....	.....	.....	0.91	.....	0.05	.....
	.....	.....	.....	100.00	.....	.....	.....	100.00

EXTRACTION WITH DILUTE ALCOHOL AFTER EXTRACTING THE FLOUR WITH 10 PER CENT SODIUM-CHLORIDE SOLUTION.

Four kilograms of "straight flour" were extracted with 10 per cent sodium-chloride solution as long as anything was removed. After squeezing as dry as possible in a screw-press, the residue was treated with alcohol so as to yield with the water retained by the meal as nearly as possible a

solution containing 75 per cent of alcohol. After digesting 2 days with the solvent, the extract was squeezed out in a press and the process repeated three times. Four extracts were thus obtained. These were each concentrated to small volume, cooled, and the solution decanted from the precipitated mass. This was then washed with distilled water. After removing the salts, the substance from extracts 1 and 2 dissolved to some extent; that from extract 3 dissolved completely to a turbid solution. By adding sodium chloride the dissolved protein was precipitated.

The residues remaining after washing with water were treated with absolute alcohol and digested with ether. The precipitates obtained from the water washings by adding salt were treated in the same way. From extract 1 preparation 72 was obtained, weighing 82 grams; extract 2, preparation 73, weighing 57 grams; from extract 3, after dissolving in water and precipitating with sodium chloride, preparation 74, weighing 11.3 grams; from extract 4 preparation 75, weighing only 1.35 grams, and from the united water washings of 72 and 73 preparation 76, weighing 5.8 grams. The total weight of these preparations was 157.45 grams, equal to 3.94 per cent of the flour taken. Their composition is shown by the analyses given in table 14 on the preceding page.

Extraction of Gluten with Dilute Alcohol.

Two kilograms of "straight spring-wheat flour" were made into a dough with distilled water of 20°, and then washed in a stream of river water of 5°. After washing until nearly all the starch was removed, the gluten was chopped up fine and digested with alcohol of 0.90 sp. gr. at a temperature of about 20°. This extraction was continued with repeatedly renewed portions of alcohol of the same strength as long as anything was removed. The extracts were united, filtered perfectly clear, and concentrated to about one-fourth their original volume. The residual solution was then cooled and allowed to stand over night to deposit the separated gliadin. The supernatant solution was poured off and the large amount of protein which had separated was then dehydrated by treatment with absolute alcohol. The decanted mother-liquor from which this protein had separated, and also the strong alcoholic solution which resulted from dehydrating the precipitated mass, were each precipitated by adding a little sodium-chloride solution. The three products thus obtained were united, digested with fresh quantities of absolute alcohol in order to complete the dehydration, and then extracted with absolute ether. Dried over sulphuric acid, the preparation weighed 82 grams, and formed, therefore, 4.10 per cent of the flour taken. Dried at 110°, this substance had the composition shown under the head "Preparation 77" in the table on page 60.

Thirty grams of preparation 77 were then dissolved in alcohol of 0.90 sp. gr., and the clear solution evaporated to small volume, cooled, and, as no protein separated, strong alcohol was added until a considerable precipitate resulted, equal to about one-half the dissolved protein. This precipitate, which, if the extracted protein were a mixture, as stated by Ritthausen, would contain the bulk of the substances insoluble in strong alcohol, weighed 12 grams. This was marked preparation 78.

The solution from which this substance had separated must have contained the chief part of the protein called by Ritthausen gluten-fibrin. It was then concentrated to small volume, cooled, water added until a considerable precipitate resulted, the solution then heated until all dissolved, and, after cooling, the mother-liquor was decanted from the separated protein. This process was repeated four times, and the precipitate finally obtained dehydrated with absolute alcohol and digested with ether. This preparation, 79, weighed 1.6 grams.

Preparations 77, 78, and 79.

	Preparation 77.		Preparation 78.		Preparation 79.	
	I.	Ash-free.	I.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.33	52.58	52.82	52.68	52.82	52.84
Hydrogen ..	6.63	6.67	6.77	6.78	7.18	7.18
Nitrogen ...	17.57	17.65	17.62	17.65	17.57	17.57
Sulphur	1.08	1.08	1.09	1.09	.....	22.41
Oxygen	.....	22.02	.....	21.80	.....	
Ash	0.50	.....	0.19	.....	0.04	.....
	.....	100.00	.....	100.00	.....	100.00

It is clear from these analyses that no separation into proteins of differing composition had thus been effected.

Extraction of "Shorts" with Dilute Alcohol.

Two kilograms of "shorts" from the spring-wheat flour were extracted with alcohol of 0.90 sp. gr. and the extract squeezed out with a screw-press. The extract, which was a deep red-brown in color, was filtered perfectly clear, and then concentrated by distillation to about one-third. On cooling, the protein separated, leaving the mother-liquor as a deep coffee-brown liquid. This was decanted, the precipitate dissolved in alcohol of 0.90 sp. gr., and again precipitated by concentration and cooling; the strongly

colored mother-liquor was decanted and this process repeated. The precipitated protein was then again dissolved in a little dilute alcohol, and the resulting solution poured into absolute alcohol, thereby precipitating the greater part of the protein and leaving the alcohol strongly colored. The precipitate was thus freed from a very considerable part of its coloring matter. After digestion with absolute alcohol, and finally with ether, this preparation, 80, was dried and analyzed with the result shown in the table below:

Owing to the fact that this preparation, 80, was still contaminated with coloring matter, and also showed slight differences in composition from the protein extracted by similar treatment from the flour, it was subjected to further treatment with a view to its more complete purification.

A part of the preparation was dissolved in 150 cc. of alcohol of 0.90 sp. gr. and the solution poured into 1000 cc. of absolute alcohol. This produced a turbid liquid, which, on adding a drop or two of sodium-chloride solution, gave a heavy precipitate that rapidly settled, leaving the alcohol colored yellow. This precipitate was again dissolved in diluted alcohol, and precipitated by pouring into ether in order to remove anything soluble in this liquid. No coloring matter was thus removed. The precipitate was then digested with absolute alcohol, yielding preparation 81.

The strong alcoholic solution from which this preparation had separated on longer standing deposited a small amount of substance which, when dehydrated, yielded preparation 82. Analysis showed these two substances to have the composition shown in the following table:

Preparations 80, 81, and 82.

	Preparation 80.		Preparation 81.				Preparation 82.			
	I.	Ash-free.	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon....	52.75	53.25	52.57	52.52	52.55	52.85	52.43	52.56	52.50	52.74
Hydrogen.	6.96	7.02	6.74	6.80	6.77	6.81	6.79	6.88	6.84	6.87
Nitrogen..	17.22	17.38	17.39	.....	17.39	17.48	17.59	.....	17.59	17.67
Sulphur...	1.36	1.37	}	.....	.....	22.86	.....	.....	.....	22.72
Oxygen...	.....	20.98								
Ash.....	0.95	.....	0.54	.....	.....	.....	0.46	.....	.....	.....
	.....	100.00	.....	.....	.....	100.00	.....	.....	.....	100.00

These figures show that the protein extracted from the "shorts" has the same composition as that similarly obtained from the flour.

PROTEIN EXTRACTED BY ALCOHOL FROM WHOLE-WHEAT FLOUR.

As Ritthausen and probably others used whole-wheat flour in extracting the various proteins described by them as soluble in dilute alcohol, it was thought best to carry out some extractions with meal obtained by grinding the entire wheat kernel in the laboratory.

Preparations 83–88.

	Preparation 83.				Preparation 84.			
	I.	II.	Aver-age.	Ash-free.	I.	II.	Aver-age.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.88	52.68	52.78	52.90	52.59	52.73	52.66	52.89
Hydrogen	7.00	6.97	6.98	6.99	6.87	6.80	6.84	6.87
Nitrogen	17.47	17.48	17.48	17.52	17.92	18.06	17.99	18.06
Sulphur	1.43	.....	1.43	1.43	0.92	.....	0.92	0.92
Oxygen	.....	.....	.....	21.16	.....	.....	.....	21.26
Ash	0.23	.....	.....	.....	0.45	.....	.....	.....
	.....	.....	.....	100.00	.....	.....	.....	100.00

	Preparation 85.				Preparation 86.	
	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	51.03	50.93	50.98	53.16	52.64	52.82
Hydrogen	6.59	6.50	6.55	6.83	6.86	6.88
Nitrogen	16.98	17.08	17.03	17.75	17.49	17.55
Sulphur	0.92	.....	0.92	0.96	.....	22.75
Oxygen	.....	.....	.....	21.30		
Ash	4.11	4.03	4.07	.....	0.35	.....
	.....	.....	.....	100.00	.....	100.00

	Preparation 87.		Preparation 88.			
	I.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.66	52.68	52.03	51.88	51.96	52.24
Hydrogen	6.80	6.81	6.66	6.69	6.68	6.71
Nitrogen	17.62	17.63	17.48	.....	17.48	17.57
Sulphur	.....	22.88	1.08	.....	1.08	1.08
Oxygen			.....	.....	.....	22.40
Ash	0.04	.....	0.52	.....	.....	.....
	.....	100.00	.....	.....	.....	100.00

One kilogram of whole spring-wheat flour, freshly ground, was made into a dough, and the gluten obtained from this by washing with water was then chopped fine and thoroughly extracted with alcohol of 0.90 sp. gr., the yellow extract concentrated, and the protein separated by cooling. The deposit thus produced was dissolved as far as possible in dilute alcohol, and the insoluble substance, which was coagulated protein, was washed with dilute alcohol, absolute alcohol, and ether. This was preparation 83.

The solution filtered from 83 was poured into absolute alcohol and a small amount of protein separated; this was treated with absolute alcohol and ether in the usual way, yielding preparation 84. The filtrate from 84 was concentrated to small volume and poured into absolute alcohol, whereby nearly all the protein was precipitated. This substance was dehydrated with absolute alcohol and digested with ether, giving preparation 85. These three bodies had the composition shown by the figures for preparations 83, 84, and 85 in the table on page 62.

In a similar manner an extract was made of winter-wheat meal obtained by grinding the entire wheat kernel in the laboratory, the alcoholic extract concentrated to about one-third of its volume, cooled, and the solution decanted from the deposit. This was then dissolved in alcohol of 0.90 specific gravity and the coagulated protein filtered off, washed with dilute alcohol, digested with absolute alcohol and then with ether, giving preparation 86.

The solution filtered from this preparation was concentrated to small volume, cooled, and the protein separated was digested with absolute alcohol and with ether, yielding preparation 87. (See table on p. 62.) The complete extraction of this protein from the gluten is very difficult, a little generally remaining in the insoluble residue after extracting with dilute alcohol. In one case the residue thus remaining was dissolved in 0.2 per cent potassium-hydroxide solution, and the resulting solution, after standing some time to deposit suspended impurities, was decanted and precipitated with dilute hydrochloric acid. This precipitate was washed by decantation with water and then digested for some time with dilute alcohol. The alcoholic solution was then filtered and concentrated to small volume and cooled. The protein separated was then digested with absolute alcohol and with ether and dried at 110° for analysis.

From this analysis it is seen that the protein soluble in dilute alcohol is not changed in composition by solution with potassium hydroxide, nor is its solubility altered, so far as could be learned.

In order to facilitate a comparison of these analyses they have been brought together in table 15.

TABLE 15.—*Composition of protein extracted by dilute alcohol.*

		Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.	Total.
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
By direct extraction.....	58[1]	.....	.....	17.18	.....	.....	.....
	59[1]	.....	.....	17.26	.....	.....	.....
	60	52.52	6.78	17.64	1.08	21.98	100
	61	52.77	6.78	17.77	1.26	21.42	100
	62	52.67	6.70	17.66	1.22	21.75	100
	63	52.55	6.85	17.94	1.21	21.45	100
	64	52.74	6.77	17.62	1.23	21.64	100
	65	52.39	6.93	17.31	1.38	21.99	100
	66	52.84	6.81	17.67	1.11	21.57	100
	68	53.02	6.79	17.32	1.05	21.82	100
	69	.....	.....	17.69	.....	.....	.....
	70	52.33	6.91	17.70	23.06		100
	71	52.38	7.13	17.82	22.67		100
After extraction with 10 per cent sodium - chloride solution..	72	52.69	6.84	17.73	1.02	21.72	100
	73	52.72	6.86	17.89	0.95	21.58	100
	74	52.71	6.81	17.75	1.10	21.63	100
	76	52.65	6.83	17.79	1.08	21.65	100
From gluten, spring - wheat flour.....	77	52.58	6.67	17.65	1.08	22.02	100
	78	52.68	6.78	17.65	1.09	21.80	100
	79	52.84	7.18	17.57	22.41		100
From gluten, whole spring-wheat flour...	83	52.90	6.99	17.52	1.43	21.16	100
	84	52.89	6.87	18.06	0.92	21.26	100
	85	53.16	6.83	17.75	0.96	21.30	100
	88	52.24	6.71	17.57	1.08	22.40	100
From gluten, whole winter-wheat flour...	86	52.82	6.88	17.55	22.75		100
	87	52.68	6.81	17.63	22.88		100
From wheat "shorts".....	80	53.25	7.02	17.38	1.37	20.98	100
	81	52.85	6.81	17.48	22.86		100
	82	52.74	6.87	17.67	22.72		100
Average of preceding figures.....		52.72	6.86	17.66	1.14	21.62	100

[1] Omitted in average.

THE PROPORTION OF GLUTAMINIC ACID YIELDED BY VARIOUS FRACTIONS OF THE ALCOHOL-SOLUBLE PROTEIN.

The results of this extensive fractionation of the alcohol-soluble protein give no evidence, based on the ultimate composition of the many fractions, of the presence of more than one protein substance. This, however, does not justify the conclusion that only one such substance exists. In view of the recent positive statements of Kossel & Kutscher and of König & Rintelen it is important to obtain, if possible, further evidence based on the proportion of some of the decomposition products yielded by the various fractions of this protein. As gliadin yields a relatively large amount of glutaminic

acid when decomposed by boiling with strong hydrochloric acid, determinations of the quantity of glutaminic acid obtained from various fractions have been made.

The first difficulty encountered in examining the evidence that has been offered respecting the existence of several alcohol-soluble proteins in wheat flour lay in the impossibility of following Ritthausen's directions for preparing these, since many evidently important details are omitted in the description of his methods.

Kossel & Kutscher state that their products were made according to Ritthausen's directions, but give no details, nor do they state which method they employed. Kutscher concludes another paper by the statement that "the wheat gluten consists of gluten-casein wholly insoluble in cold 60 per cent alcohol; gluten-fibrin, but little soluble, and gliadin, easily soluble in cold 60 per cent alcohol."

Although the writer has made a very large number of preparations representing fractions of this protein substance dissolved by alcohol of various degrees of strength, he has never obtained any that were not either completely soluble in cold alcohol of 60 per cent by volume, or else contained such insignificant quantities which did not dissolve that he has found it impossible to make from them a preparation of "gluten-fibrin" suitable for further examination. He has, therefore, been unable to repeat the work of Kossel & Kutscher and is entirely at a loss to understand how their preparation of "gluten-fibrin" was obtained.

König & Rintelen describe their procedure in more detail. These investigators extracted wheat gluten with absolute alcohol, added ether to the alcoholic extract, and united the precipitate produced with the extracted gluten. This latter was then extracted with 65 per cent alcohol, and to the extract alcohol was added until the mixture contained 88 to 90 per cent of alcohol.

After decanting from the precipitate that had formed, the solution was filtered clear and evaporated to dryness on the water-bath, finely pulverized, and extracted with ether to remove fat. As all of the fat could not be thus removed, the mass was again dissolved in alcohol, to which some potassium hydroxide was added, and this solution shaken out several times with ether. The weakly alkaline solution was then exactly neutralized with hydrochloric acid and evaporated on the water-bath. The product thus obtained was their "gluten-fibrin."

The precipitate produced by 88 to 90 per cent alcohol was washed with alcohol of the same strength and dissolved in a little 65 per cent alcohol. From this one-half of the alcohol was distilled off and the residual solution cooled, when a precipitate separated. From this the solution was decanted, leaving a mass of gliadin. The solutions which remained from several such

precipitates were united and distilled until one-third of the solvent was removed. On cooling the residual solution, a deposit formed which they considered to be a mixture of gliadin and mucedin. The solution decanted from this deposit was evaporated to dryness, and yielded a considerable residue of "mucedin."

The analyses of these products showed that the gliadin thus prepared had the same composition as that obtained by the writer, as well as that made by Ritthausen, while the "gluten-fibrin" and mucedin contained about 1 per cent less nitrogen and much more carbon.

König & Rintelen obtained their mucedin from the nearly aqueous solutions remaining after separating the gliadin by evaporating to dryness. The writer assumes that the residue which remained was subjected to some further purification, but concerning this they say nothing. The solution contains many impurities, and when the protein substance in it has been properly purified it has the properties and composition of gliadin.

Although large quantities of gliadin have been made in this laboratory and subjected to very careful and extensive fractionation, no evidence whatever of the existence of "mucedin" has been obtained.

Furthermore, it would seem improbable that our gliadin could be contaminated by "gluten-fibrin" and "mucedin," which the writer certainly did not succeed in separating from it, and at the same time show so close an agreement in composition with that of König & Rintelen, from which they suppose that both of these proteins had been carefully removed. It is also improbable that Kjeldahl should have found the specific rotation uniform for numerous preparations of the alcohol-soluble protein if the material which he examined was a mixture of three different substances; nor, if this were the case, could his determination of $(\alpha)_D$ —92° be expected to agree so closely with that made by Osborne & Harris,[1] $(\alpha)\frac{20°}{D}$ —92.3°, and by Mathewson,[2] $(\alpha)\frac{40°}{D}$ —91.95°.

The composition of both "gluten-fibrin" and "mucedin" differs from that of gliadin just as one would expect if these former substances were slightly altered, and somewhat impure products obtained from gliadin.

Until more convincing evidence of the existence of "gluten-fibrin" and "mucedin" as distinct protein substances is brought forward, they can not be considered to be original constituents of the wheat kernel.

Kutscher has stated that there is a wide difference in the proportion of glutaminic acid which is yielded by gliadin and gluten-fibrin. The writer

[1] Osborne & Harris, Journal American Chemical Society, 1903, XXIV, p. 844.
[2] Mathewson, Journal American Chemical Society, 1906, XXXVIII, p. 1482.

has therefore determined the amount of this amino-acid which was yielded by several preparations of the alcohol-soluble protein, one of which represented the fraction of the protein soluble in the strongest alcohol, and should therefore, according to Ritthausen, consist chiefly of gluten-fibrin. The amount of glutaminic acid in gliadin was first determined by boiling 100 grams of gliadin, equal to 93 grams moisture free, for 14 hours with 200 cc. of concentrated hydrochloric acid. After standing on ice for 3 days, the entire solution solidified to a thick mass of crystals, which was sucked out with a pump and washed with ice-cold alcoholic hydrochloric acid. When dried over sodium hydroxide, this crude glutaminic acid hydrochloride weighed 58.33 grams. The filtrate and washings on concentration gave by similar treatment 2.58 grams more, making the total crude glutaminic acid hydrochloride 60.01 grams. This product was then dissolved in water freed from color with animal charcoal and recrystallized. After removing ammonium chloride by boiling with a slight excess of barium hydroxide and the barium with an equivalent quantity of sulphuric acid, 43.02 grams of pure glutaminic acid hydrochloride were obtained, which are equal to 34.42 grams of the free acid, or 37 per cent of the gliadin.

Nitrogen: 0.5092 gram substance, dried at 100°, gave $NH_3 = 3.86$ HCl (1 cc. HCl = 0.01 gram N) = 7.58 p. ct. N.

Calculated for $C_5H_{10}O_4NCl$, 7.64 p. ct. N.

In confirmation of these figures this determination was repeated with two fractions of the alcohol-soluble protein of wheat gluten which had been separated from relatively strong alcoholic solutions, and should therefore have contained a large proportion of "gluten-fibrin" if the statements respecting the solubility of this substance are correct.

Two portions of different preparations of the air-dry substance, equivalent to 18.62 and 14.65 grams dried at 110°, were hydrolyzed as before, their solutions saturated with hydrochloric acid, and kept for some time on ice. The glutaminic acid hydrochloride which separated was filtered out, washed with alcoholic hydrochloric acid, and freed from ammonia by evaporating with an excess of baryta and from barium by an equivalent quantity of sulphuric acid. The solution was then decolorized with animal charcoal and evaporated with an excess of hydrochloric acid until crystallization began. The glutaminic acid hydrochloride which separated, when washed with ice-cold alcoholic hydrochloric acid and dried, weighed, respectively, 8.69 and 6.27 grams, equivalent to 37.33 and 34.2 per cent of free glutaminic acid in the protein.

Another attempt was made to isolate a fraction of "gluten-fibrin" by dissolving 200 grams of a preparation representing the total alcohol-soluble protein of wheat gluten in a mixture of 900 cc. of absolute alcohol and

600 cc. of water—that is, in alcohol of 60 per cent by volume. Although the solution was somewhat turbid, nothing was deposited, even on long standing. 300 cc. of water were then added to the solution, making the alcohol 50 per cent, but still nothing separated. The strength of the alcohol was therefore raised to 75 per cent by adding 1800 cc. of absolute alcohol, and a very large precipitate, A, at once separated. By adding 2000 cc. of absolute alcohol to the clear solution from which A separated another large precipitate (B) was produced, and in the filtrate from B a third precipitate (C) resulted, when a further large quantity of absolute alcohol was added. This last product weighed 20 grams and constituted only 10 per cent of the total protein. The solution from which C separated contained only traces of protein, and C, therefore, represented the fraction of the whole protein soluble in the strongest alcohol, and should consequently contain much "gluten-fibrin." In 17.5 grams of this substance dried at 110° the glutaminic acid produced by decomposing with hydrochloric acid was determined. By proceeding in the same manner as in the experiment last described 7.8914 grams of pure glutaminic acid hydrochloride were obtained, which are equivalent to 6.2131 grams of the free acid, or 35.5 per cent.

Nitrogen: 0.7296 gram substance, dried at 110°, gave $NH_3 = 5.66$ cc. HCl (1 cc. HCl = 0.0100 gram N) = 7.75 p. ct. N.
Calculated for $C_5H_9NO_4HCl$, 7.64 p. ct. N.

This result seems to furnish conclusive evidence that there is no fraction, soluble in very strong alcohol, to be obtained from the alcohol-soluble protein of wheat gluten that is characterized by yielding a relatively small proportion of glutaminic acid.

Since Kutscher decomposed his proteins with sulphuric acid, while hydrochloric acid was used in the preceding experiments, the following experiment was made in order to determine whether the higher yield obtained by us might not be due to this fact. Accordingly 50 grams of one of the preparations of gliadin, from which 37 per cent of glutaminic acid had previously been isolated, were boiled for 14 hours with a mixture of 150 grams of sulphuric acid and 300 cc. of water.

The resulting solution was treated with an excess of baryta and the ammonia expelled by evaporation. The barium was then removed by an equivalent amount of sulphuric acid and the filtered solution evaporated. Some tyrosine separated, which was filtered out, and the evaporation continued until the volume was quite small. On standing, an abundant quantity of crystals of free glutaminic acid separated, and from the mother-liquor, by further concentration and standing, a second crop of crystals was obtained. After recrystallizing several times, 8.48 grams of pure glutaminic acid were obtained, which contained 9.43 per cent of nitrogen.

Nitrogen: 0.6094 gram substance, dried at 110°, gave $NH_3 = 5.75$ cc. HCl (1 cc. HCl = 0.0100 gram N) = 9.43 p. ct.; calculated for $C_5H_9NO_4$, 9.48 p. ct.

From the mother-liquors which yielded this glutaminic acid there were further separated, by saturating with hydrochloric acid and proceeding in the manner already described, 4.055 grams of well-crystallized hydrochloride, which contained 7.73 per cent of nitrogen.

Nitrogen: 0.4983 gram substance, dried at 110°, gave $NH_3 = 3.85$ cc. HCl (1 cc. HCl = 0.0100 gram N) = 7.73 p. ct. N; calculated for $C_5H_9NO_4HCl$, 7.64 p. ct. N.

The free glutaminic acid corresponding to this amount of the hydrochloride is 3.244 grams, making a total of 11.724 grams. Since the 50 grams of air-dry gliadin were equivalent to 46.33 grams dried at 110°, this amount of glutaminic acid is equal to 25.3 per cent, or about the same proportion as Ritthausen found in his preparation of "mucedin" after decomposing with sulphuric acid, but much more than the 19.81 per cent found by Kutscher in the same substance. The amount, however, was only about two-thirds as much as that found after decomposing with hydrochloric acid, and although the separation was not complete there was no reason to suppose that more remained in the mother-liquors in one case than in the other. It is possible, however, that the very large precipitate of barium sulphate that formed on removing the sulphuric acid retained a considerable part of the glutaminic acid even after extensive washing with hot water.

In conclusion, the results of these determinations are here brought together that they may be more readily compared:

TABLE 16.—*Percentage of glutaminic acid yielded by gliadin.*

Preparation.	Decomposed by —	
	HCl.	H_2SO_4.
	P. ct.	*P. ct.*
1	37.00	.....
2	37.33	.....
3	34.20	.....
4	35.50	.....
5	.....	25.3

All these are minimal figures, since in each case some glutaminic acid still remained in the mother-liquors, but it does not seem probable that more than relatively insignificant quantities were thus lost.

From these results it would appear that Kutscher's determinations of glutaminic acid fall far short of the actual quantity of this substance yielded by the alcohol-soluble protein of wheat, and that they therefore afford no

evidence which justifies the conclusion that this substance consists of two distinct protein bodies. Fractional precipitations of this alcohol-soluble protein yield practically the same large proportion of glutaminic acid, so that, in view of their very close agreement in composition and properties, both physical and chemical, there is every reason to believe that only one such protein is present. Gliadin yields a remarkable proportion of glutaminic acid, much in excess of that from any other known protein and greater than that of any single decomposition product yet obtained in a pure state from any other true protein substance, the protamines, of course, excepted.

HYDROLYSIS OF GLIADIN.

There being no sufficient evidence that more than one alcohol-soluble protein occurs in the wheat kernel, no attempt has been made, in preparing large quantities of gliadin for the present investigation, to subject the protein matter extracted by alcohol to any fractional precipitation, but it was separated as completely as possible from all other substances soluble in water, alcohol, and ether.

The gliadin for this investigation was prepared entirely from gluten, as thereby the water-soluble constituents of the seed are more completely removed than by any other method of preparation which can be readily used on a large scale. The wheat flour was kneaded into dough in a domestic "bread-mixer," and then under water in a specially constructed kneading-machine. After frequently decanting and renewing the water, a thoroughly coherent gluten was obtained. This was washed practically starch free in a current of water and, while moist, was ground by passing through a special form of "drug-press," which was a ready means of reducing it to comparatively small pieces. The ground gluten was then extracted with alcohol of such strength that, with the combined water of the gluten, a solvent of 60 to 70 per cent by volume resulted. The extracts were filtered *perfectly* clear through thick felts of filter paper pulp, and the water-clear solution, free from any trace of opalescence or turbidity, was evaporated to a small volume on a water-bath. The thick sirup that resulted was cooled and then poured, with constant and rapid stirring, into a large volume of distilled ice-water containing a very little sodium chloride. The gliadin was thus precipitated as a filament, which, on stirring, united to a coherent plastic mass. This gliadin was next dissolved by stirring with strong alcohol until all had gone into solution, the water combined with the precipitated gliadin being sufficient to dilute the alcohol to the proper degree. The resulting solution was evaporated to a thick sirup, absolute alcohol being added from time to time in order to hold the gliadin in the solution, since this, during the evaporation, became constantly more aqueous. The thick

sirup was then poured, in a very fine stream, into a large volume of absolute alcohol under rapid and constant stirring. In this way a porous mass of protein was obtained which was at once reduced to small pieces and digested under fresh absolute alcohol. When well dehydrated, the gliadin was digested with ether, partially dried over sulphuric acid, ground to a coarse powder, and then dried thoroughly over sulphuric acid. When thus prepared, gliadin forms a snow-white, friable mass which is easily reduced to a powder.

1100 grams of gliadin, equal to 998.6 grams dried at 110°, were heated with a mixture of 1000 cc. of concentrated hydrochloric acid and 1000 cc. of water on the water-bath for several hours, until the gliadin had dissolved and frothing had ceased. The solution was boiled in an oil-bath having a temperature of 115° for 10 hours, cooled with ice, and saturated with gaseous hydrochloric acid. After remaining on ice for 2 days, the glutaminic acid hydrochloride that had separated was filtered out, washed with ice-cold alcoholic hydrochloric acid dissolved in water, the solution treated with bone-black and freed from ammonia by boiling with an excess of barium hydroxide. After removing the barium with an equivalent amount of sulphuric acid, the glutaminic acid was separated as hydrochloride, and, when recrystallized and thoroughly dried, weighed 374.3 grams, equivalent to 300 grams of free glutaminic acid. This was converted into the free acid, which melted at 202° to 203°.

Carbon and hydrogen: 0.6218 gram substance gave 0.9276 gm. CO_2 and 0.3494 gm. H_2O.
Nitrogen: 0.4574 gram substance gave NH_3 = 4.3 cc. HCl (1 cc. HCl = 0.01 gram N).
Calculated for $C_5H_9O_4N$, C 40.78, H 6.18, N 9.54 p. ct.; found, C 40.69, H 6.24, N 9.40 p. ct.

The mother-liquor from the recrystallized glutaminic acid was added to the filtrate from the first separation of the glutaminic acid, and the entire solution concentrated to a sirup under strongly reduced pressure. 3 liters of alcohol, previously saturated with hydrochloric acid at a low temperature, were added to the sirup, and dry hydrochloric acid gas was passed into the solution until it was saturated. The mixture was again concentrated as before, under reduced pressure, the sirup again taken up in 3 liters of alcoholic hydrochloric acid, and after standing several hours again concentrated to a sirup, taken up a third time in alcoholic hydrochloric acid, and after some hours concentrated to a sirup on a bath of 40° and under a pressure of from 5 to 10 mm. The neutralization, extraction, and drying of the esters were conducted according to the method described by Emil Fischer.[1]

The undistilled residue, after distillation A, weighed 180 grams.

The residue which remained after extracting the esters with ether was made strongly acid with hydrochloric acid, freed from sodium and potassium

[1] Fischer, Emil, Zeitschrift für physiologische Chemie, 1901, XXXIII, p. 151.

salts by repeated evaporations with alcoholic hydrochloric acid and thorough extraction of the precipitated chlorides with the latter. The alcoholic solution of the chlorides of the amino-acids was evaporated to a sirup, and esterification repeated as in the first instance. After extracting the esters with ether and drying them, the entire process was repeated, and the ethereal solution of the esters resulting from this third treatment was united with that from the second, thus following the method applied by Abderhalden to oxyhemoglobin.[1]

Distillations A and B.

Fraction.	Distillation A.				Distillation B.		
	Temperature of bath up to—	Vapor.	Pressure.	Weight.	Temperature of bath up to—	Pressure.	Weight.
	°	°	*mm.*	*Grams.*	°	*mm.*	*Grams.*
I.....	93	....	12.0	28.18	83	12.0	20.13
II.....	100	75–76	12.0	47.03	100	12.0	36 81
III.....	120	....	0.8	64.68	120	0.8	62.70
IV.....	160	....	0.8	40.00	200	0.8	33.00
	.....	.....	.....	179.89	.....	.....	152.64

Fraction I.

Distillation A.—This was saponified at once by evaporation with concentrated hydrochloric acid on a water-bath, the residue taken up in alcohol, the solution saturated with dry hydrochloric acid gas, and a crystal of glycocoll ester hydrochloride added. After prolonged standing on ice, no separation occurred. The solution was then evaporated on the water-bath with concentrated hydrochloric acid, the latter removed with lead oxide and the lead with hydrogen sulphide. The amino-acids were subjected to fractional crystallization.

Distillation B was treated in substantially the same way, but although several attempts were made to isolate the hydrochloride of glycocoll ester, none was found. By systematic fractionation there were obtained from fraction I of the two distillations, A and B, 6.68 grams alanine and 0.86 gram leucine.

The leucine, when recrystallized from dilute alcohol, decomposed at about 298°.

Carbon and hydrogen: 0.1778 gram substance, dried at 110°, gave 0.3577 gram CO_2 and 0.1608 gram H_2O.

Calculated for $C_6H_{13}O_2N$, C 54.89, H 10.01 p. ct.; found, C 54.87, H 10.04 p. ct.

[1]Abderhalden, Zeitschrift für physiologische Chemie, 1903, xxxvii, p. 484.

The alanine, when recrystallized by dissolving in a little hot water and gradually adding alcohol, decomposed at about 290°.

Carbon and hydrogen: 0.2404 gram substance, dried at 110°, gave 0.3571 gram CO_2 and 0.1712 gram H_2O.

Nitrogen: 0.3980 gram substance gave $NH_3 = 6.2$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_3H_7O_2N$, C 40.40, H 7.93, N 15.75 p. ct.; found, C 40.51, H 7.91, N 15.58 p. ct.

{ Fraction II. Temperature of bath up to 100°.
Pressure, 12 mm. Weight, 83.84 grams. }

Distillations A and B.—Each was saponified by boiling for 5½ hours with 5 parts of water.

The solution was evaporated to dryness under reduced pressure, the dried residue boiled up with absolute alcohol, and 13.37 grams were dissolved. This solution was united with a similar one obtained from fraction III. The substance, insoluble in alcohol, after systematic fractional crystallization, gave—

(I) 22.1 grams of leucine, which decomposed at about 298° and had the following composition :

Carbon and hydrogen: 0.3128 gram substance, dried at 110°, gave 0.6310 gram CO_2 and 0.2794 gram H_2O.

Calculated for $C_6H_{13}O_2N$, C 54.89, H 10.01 p. ct.; found, C 55.01, H 9.92 p. ct.

(II) A fraction of 3.45 grams which by fractional crystallization could not be further separated and gave results on analysis which agreed best for a mixture of leucine and amino-valerianic acid.

Carbon and hydrogen: 0.2857 gram substance, dried at 110°, gave 0.5519 gram CO_2 and 0.2577 gram H_2O.

Calculated for equal molecules of leucine and amino-valerianic acid, C 53.05, H 9.74 p. ct.; found, C 52.68, H 10.02 p. ct.

(III) 2.1 grams substance which had the properties and composition of amino-valerianic acid.

Carbon and hydrogen: (I) 0.2115 gram substance, dried at 110°, gave 0.3957 gram CO_2 and 0.1870 gram H_2O; (II) 0.4597 gram substance, dried at 110°, gave 0.8643 gram CO_2 and 0.3956 gram H_2O.

Calculated for $C_5H_{11}O_2N$, C 51.22, H 9.48 p. ct.; found, (I) C 51.03, H 9.82 p. ct.; (II) C 51.27, H 9.56 p. ct.

Specific rotation.—Dissolved in 20 per cent hydrochloric acid, $(\alpha)\frac{20°}{D} = +25.79°$. E. Fischer and Dörpinghaus[1] found $+25.9°$ for their preparation from horn, and Schulze and Winterstein[2] found $+28.2°$ and $+27.9°$ for preparations from lupine seedlings.

[1] Fischer & Dörpinghaus, Zeitschrift für physiologische Chemie, 1902, XXXVI, p. 462.
[2] Schulze & Winterstein, ibid., 1902, XXXV, p. 300.

The chlorine was removed from the solution used for determining the specific rotation and the substance racemized by heating with 20 cc. of water and 7 grams of crystallized barium hydroxide for 19 hours in an autoclave at 175°. The barium was quantitatively removed with sulphuric acid, and the α-naphthyl-hydantoic acid prepared according to the directions of Neuberg & Manasse.[1] This crystallized in long needles and melted constantly on repeated recrystallization from 40 per cent alcohol at 180° to 181°.

Carbon and hydrogen: 0.3235 gram substance, dried at 90°, gave 0.7936 gram CO_2 and 0.1909 gram H_2O.

Calculated for $C_{16}H_{18}O_3N_2$, C 67.07, H 6.35 p. ct.; found, C 66.90, H 6.56 p. ct.

By racemizing the remaining mixture of undetermined amino-acids we were unable to isolate any more amino-valerianic acid.

(IV) 8.6 grams alanine. This was racemized by heating with an excess of barium hydroxide and coupled with α-naphthyliso-cyanate according to the directions of Neuberg & Manasse. The hydantoic acid, which crystallized in prisms, melted at 197°.

Carbon and hydrogen: 0.3146 gram substance, dried at 110°, gave 0.7480 gram CO_2 and 0.1560 gram H_2O.

Calculated for $C_{14}H_{14}O_3N_2$, C 65.06, H 5.48 p. ct.; found, C 64.84, H 5.51 p. ct.

{ Fraction III. Temperature of bath, up to 120°.
Pressure, 0.8 mm. Weight, 127.38 grams. }

This fraction was boiled for 5½ hours with 8 parts of water. The solution, evaporated to dryness under reduced pressure, gave 98 grams of amino-acids or 80 per cent of the esters. Of this 59.94 grams were soluble in alcohol. From the part insoluble in alcohol, by systematic fractional crystallization, there were isolated 33.06 grams of leucine and 4.57 grams of alanine. The leucine decomposed at about 298°.

Carbon and hydrogen: 0.2416 gram substance, dried at 110°, gave 0.4872 gram CO_2 and 0.2166 gram H_2O.

Nitrogen: 0.2690 gram substance gave NH_3 = 2.85 cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_6H_{13}O_2N$, C 54.89, H 10.01, N 10.70 p. ct; found, C 54.99, H 9.96, N 10.59 p. ct.

The alcohol-soluble substance from fraction II was united with that from fraction III. The solution was evaporated to dryness under reduced pressure, and the residue taken up in water and boiled gently for about an hour

[1] Neuberg & Manasse, Berichte der deutschen chemischen Gesellschaft, 1905, XXXVIII, p. 2359.

with an excess of copper hydroxide. The filtered solution was evaporated to dryness under reduced pressure and the residue boiled with absolute alcohol. The undissolved part was dissolved in water, freed from copper by hydrogen sulphide, and the solution again evaporated to dryness under reduced pressure. The residue was boiled with absolute alcohol, in which all of it dissolved. The alcohol was evaporated off under reduced pressure, the residue dissolved in 500 cc. of water and again converted into the copper salt. By concentration 19.91 grams of crystalline racemic proline copper salt were obtained, which is equal to 13.98 grams of *α*-proline. This was recrystallized from water and dried in the air.

Water: 0.7789 gram substance lost 0.0856 gram H_2O at 110°.
Calculated for $C_{10}H_{16}O_4N_2$ Cu · $2H_2O$, H_2O 11.00 p. ct.; found, H_2O 10.98 p. ct.
Carbon and hydrogen: 0.6851 gram substance, dried at 110°, gave 1.0310 gram CO_2 and 0.3450 gram H_2O.
Copper: 0.2926 gram substance gave 0.0799 gram CuO.
Calculated for $C_{10}H_{16}O_4N_2$ Cu, C 41.11, H 5.54, Cu 21.79 p. ct; found, C 41.04, H 5.59, Cu 21.81 p. ct.

The *r*-proline copper salt was freed from copper with hydrogen sulphide, its solution evaporated to dryness, and the residue recrystallized from alcohol. After drying in vacuo over sulphuric acid the *r*-proline melted at 203° to 205°.[1]

Carbon and hydrogen: 0.3373 gm. substance gave 0.6424 gm. CO_2 and 0.2453 gm. H_2O.
Calculated for $C_5H_9O_2$ N, C 52.12, H. 7.90 p. ct.; found, C 51.94, H 8.08 p. ct.

The solution of the alcohol-soluble copper salt was evaporated to dryness and left a residue of *l*-proline copper salt which, dried at 120°, weighed 71.62 grams, which is equal to 56.51 grams of free *l*-*α*-proline.

Copper: 0.2850 gram substance, dried at 110°, gave 0.0760 gram CuO.
Calculated for $C_{10}H_{16}O_4N_2$ Cu, Cu 21.79 p. ct.; found, Cu 21.30 p. ct.

One-half of this proline copper salt was freed from copper, and the proline racemized by heating with 150 cc. of water containing 80 grams of crystallized barium hydroxide for 5 hours at 150°. The barium was removed quantitatively with sulphuric acid, the solution concentrated, and the proline again converted into the copper salt. There was thus obtained 20.1 grams of very nearly pure racemic *α*-proline copper.

Water: 0.3356 gram substance, air-dry, lost 0.0369 gram H_2O at 110°.
Copper: 0.2640 gram substance gave 0.0640 gram CuO.
Calculated for $C_{10}H_{16}O_4N_2$ Cu · $2H_2O$, H_2O 11.00, Cu 19.40 p. ct.; found, H_2O 11.00, Cu 19.35 p. ct.

[1] Willstaeter, R., Berichte der deutschen chemischen Gesellschaft, 1900, XXXIII, p. 1160; Fischer, Emil, ibid., 1901, XXXIV, p. 458.

The total crystalline racemic copper salt was equal to 41.96 grams of *r*-*a*-proline.

From the other half of *l*-proline copper salt the free proline was regenerated and recrystallized from alcohol. A small part only was obtained in a crystalline condition, which melted at 205° to 206°.

Carbon and hydrogen: 0.2729 gram substance, dried over H_2SO_4, gave 0.5198 gram CO_2 and 0.1977 gram H_2O.

Nitrogen: 0.2453 gram substance gave NH_3 = 2.95 cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_5H_9O_2N$, C 52.12, H 7.90, N 12.20 p. ct.; found, C 51.95, H 8.04, N. 12.03 p. ct.

From fraction III there were isolated 4.57 grams alanine, 33.06 grams leucine, and 70.49 grams *a*-proline, including in this last that from fraction II, which was not weighed separately.

Fraction IV.	Temperature of bath to—	Pressure.	Weight.
	°	*mm.*	*Grams.*
A.....	160	0.8	39.96
B.....	200	0.8	33.00
	.....	.....	72.96

This was treated with water and shaken out with ether according to the procedure described by Emil Fischer.[1]

The ether was carefully removed by evaporation and the residual phenylalanine ester saponified by dissolving in concentrated hydrochloric acid and evaporating on a water-bath. The phenylalanine hydrochloride weighed 29.14 grams, equivalent to 23.87 grams of free phenylalanine. The phenylalanine hydrochloride was recrystallized from strong hydrochloric acid. It was decomposed by evaporating with an excess of ammonia and the phenylalanine recrystallized from water. It melted, on slow heating, at 263° to 265°.[2]

Carbon and hydrogen: 0.3051 gram substance, dried at 110°, gave 0.7322 gram CO_2 and 0.1792 gram H_2O.

Nitrogen: 0.3020 gram substance gave NH_3 = 2.53 cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_9H_{11}O_2N$, C 65.40, H 6.73, N 8.50 p. ct.; found, C 65.44, H 6.53, N 8.38 p. ct.

[1] Fischer, E., Zeitschrift für physiologische Chemie, 1902, XXXVI, p. 274.

[2] Fischer, Emil, & Abderhalden, Zeitschrift für physiologische Chemie, 1902, XXXVI, p. 268; Erlenmeyer & Lipp, Annalen der Chemie, 1883, CCXIX, p. 197.

The aqueous layer was heated with an excess of barium hydroxide on a water-bath for 5 hours. After standing some time, the barium salt that had separated was filtered out and decomposed by an equivalent amount of sulphuric acid. The solution on concentration yielded 5.76 grams of aspartic acid.

Carbon and hydrogen: 0.3866 gram substance, dried at 110°, gave 0.5109 gram CO_2 and 0.1852 gram H_2O.

Nitrogen: 0.3637 gram substance gave $NH_3 = 3.78$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_4H_7O_4N$, C 36.09, H 5.26, N 10.53 p. ct.; found, C 36.04, H 5.32, N 10.39 p. ct.

The filtrate from the barium aspartate was freed from barium, concentrated to small volume, and saturated with hydrochloric acid. On prolonged standing a trace of phenylalanine hydrochloride separated, but no glutaminic acid hydrochloride was obtained. After removing the hydrochloric acid with silver sulphate and the sulphuric acid with barium hydroxide, the solution was boiled with an excess of copper hydroxide, but no copper salt could be separated from it, even after concentrating to a very small volume. The copper was then removed and the solution treated with bone-black and, when concentrated in a vacuum over sulphuric acid, gave crystals which, on fractional crystallization from water, gave 0.42 gram of serine, which, in an open capillary, browned at about 218° and decomposed to a brownish mass at about 240.°

Carbon and hydrogen: 0.2577 gram substance, dried at 110°, gave 0.3224 gram CO_2 and 0.1602 gram H_2O.

Calculated for $C_3H_7O_3N$, C 34.24, H 6.73 p. ct.; found, C 34.12, H 6.91 p. ct.

In the filtrate from the serine there was obtained about 5.32 grams of crystalline substance, from which nothing definite could be isolated.

Distillation Residue.

The residues from distillations A and B were dissolved in boiling alcohol and the solutions united. On cooling, 5.76 grams of long hair-like crystals separated.

The filtrate from this substance was freed from alcohol and saponified by heating with 200 grams of crystallized barium hydroxide, the barium removed, the solution concentrated under reduced pressure to small volume, saturated with hydrochloric acid, and, after standing on ice for some time, yielded 74.21 grams glutaminic acid hydrochloride, equal to 59.46 grams.[1]

The glutaminic acid hydrochloride melted at about 198° with effervescence.

Carbon and hydrogen: 0.4028 gram substance, dried over H_2SO_5, gave 0.4802 gram CO_2 and 0.2056 gram H_2O.

Calculated for $C_5H_{10}O_4NCl$, C 32.67, H 5.50 p. ct.; found, C 32.51, H 5.67 p. ct.

[1] Abderhalden & Wells, Zeitschrift für physiologische Chemie, 1905, XLVI, p. 31.

Specific rotation.—Dissolved in 20 per cent hydrochloric acid, $(\alpha)\ \frac{20^\circ}{D} = +31.47^\circ$.

Fischer & Dörpinghaus found $+31.91^\circ$ for a preparation from horn, $+30.45^\circ$ from gelatin, and $+28.20^\circ$ for one from casein.[1]

The residue which remained after removing the esters with ether from the original solution of the products of hydrolysis was treated in the way described by Emil Fischer[2] for the isolation of oxy-proline. The only substance, however, that could be isolated was serine, of which 0.87 gram was obtained, which browned at about 219° and decomposed at about 240°.

Carbon and hydrogen: 0.2987 gram substance, dried at 110°, gave 0.3743 gram CO_2 and 0.1844 gram H_2O.

Calculated for $C_3H_7O_3N$, C 34.24, H 6.73 p. ct.; found, C 34.18, H 6.85 p. ct.

In the filtrate from this serine β-naphthalene-sulphone chloride failed to give any definite product.

There were thus isolated in the two distillations from fraction I, 6.68 grams; from fraction II, 8.59 grams, and from fraction III, 4.57 grams of alanine, or 19.84 grams in all; from fraction II, 2.1 grams of amino-valerianic acid; from fraction I, 0.86 gram; from fraction II, 22.10 grams; from fraction III, 33.06 grams of leucine—in all 56.02 grams; from fractions II and III, 70.49 grams of proline; from fraction IV, 23.47 grams of phenyl-alanine, 5.76 grams of aspartic acid, and 0.42 grams of serine, and from the residue which remained after extracting the esters 0.87 gram of serine, or 1.29 grams in all.

From the main solution of the total products of hydrolysis there were obtained 300 grams of glutaminic acid, and from the residues after distilling the esters 59.5 grams, or in all 359.5 grams.

As the amount of proline found in this hydrolysis was so great, the result was confirmed by a second hydrolysis, and another effort made to obtain glycocoll, which, if present in very small amount, might have escaped detection.

For this purpose 500 grams of gliadin, air-dry, equal to 439.6 grams dried at 110°, were hydrolyzed in the same way as in the preceding hydrolysis. After esterifying and shaking out the esters three times, as before, the ether was removed by distillation at 760 mm. and the esters distilled.

[1] Cf. Fischer, Emil, & Dörpinghaus, Zeitschrift für physiologische Chemie, 1902, XXXVI, p. 475.

[2] Fischer, Emil, Berichte der deutschen chemischen Gesellschaft, 1902, XXXV, p. 2660

Fraction.	Temperature of bath up to—	Pressure.	Weight.
	°	*mm.*	*Grams.*
I.....	95	17.00	20.20
II.....	80	5.00	46.08
III..... A	110	2.00	44.14
III..... B	110	0.88	15.70
IV.....	180	0.88	54.70
V.....	200	0.78	29.69
	.....	.....	210.51

Fraction I was immediately evaporated on the water-bath with hydrochloric acid, the residue dissolved in alcohol, and the solution saturated with dry hydrochloric acid gas. The solution was concentrated to a small volume at a low temperature under a pressure of 10 mm., the residue taken up in alcohol, its solution cooled to 0° and saturated with hydrochloric acid gas. On prolonged standing 0.22 gram of glycocoll ester hydrochloric separated, which melted at 144° to 145°. When mixed with pure glycocoll ester hydrochloride, the melting-point was unchanged.

Chlorine: 0.1058 gram substance gave 0.1063 gram AgCl = Cl 24.86.
Calculated for $C_4H_{10}O_2NCl$, Cl 25.40 p. ct

Neither fraction II nor the ether distilled from the esters gave evidence of glycocoll.

This preparation of gliadin did, in fact, contain a very small amount of glycocoll, which is possibly due to a slight contamination with glutenin, in which there has since been found a notable quantity of this amino-acid.

Fraction II was saponified, and the solution evaporated under highly reduced pressure from a bath at 40°, and the residue extracted with alcohol, in which about 18 grams dissolved.

Fraction III, by similar treatment, yielded 24 grams of alcohol-soluble substance. The alcoholic solutions were united and evaporated to dryness from a bath at 40°. The crystalline residue, when dried to constant weight in vacuo, weighed 39.59 grams. From this, by extraction with alcohol, 8.7 grams of substance insoluble therein were separated.[1] The total proline thus found was 30.89 grams, equal to 7.03 per cent.

[1] Emil Fischer employs this method for estimating the proportion of proline in proteins, but stated that the result obtained is too high (Berichte der deutschen chemischen Gesellschaft, 1906, XXXIX, p. 530).

CYSTINE.

300 grams of gliadin were digested for 2 to 3 hours at 85° with 900 cc. of hydrochloric acid, sp. gr. 1.19, and the solution boiled for 3 hours. This was then concentrated to a sirup under diminished pressure, diluted to 900 cc. with cold water, and neutralized with 50 per cent sodium-hydroxide solution. After boiling with a large amount of bone-black and concentrating to 800 cc., much substance separated, which was recrystallized from about 300 cc. of water. The recrystallized product was dissolved in 5 per cent sulphuric acid and precipitated by mercuric sulphate solution.[1]

The mercury precipitate was decomposed by hydrogen sulphide, the solution freed from hydrogen sulphide, neutralized with sodium hydroxide, and acidified with acetic acid. On standing, cystine separated from the solution in hexagonal plates, and by adding alcohol to the filtrate more was obtained. When no more cystine could be thus obtained, the precipitation with mercuric sulphate was repeated. By several repetitions of this process 1.18 grams of cystine were finally isolated, which, when recrystallized by dissolving in dilute ammonia and acidifying with acetic acid, gave the following analysis:

Carbon and hydrogen: 0.3063 gram substance, dried at 110°, gave 0.3379 gram CO_2 and 0.1444 gram H_2O.

Calculated for $C_6H_{12}O_4N_2S_2$, C 29.96, H 5.04 p. ct.; found, C 30.08, H 5.23 p. ct.

TYROSINE.

219 grams of gliadin, equal to 200 grams dried at 110°, were treated with 600 cc. of concentrated hydrochloric acid, digested for some time on a water-bath, and the solution boiled for 12 hours on an oil-bath. The solution was freed from most of the glutaminic acid by saturating with hydrochloric acid, and the filtrate from the glutaminic acid was diluted, boiled with bone-black, and then concentrated strongly to remove as much hydrochloric acid as possible. The rest of the acid was neutralized with 50 per cent sodium-hydroxide solution. On standing, a considerable precipitate separated, which was filtered out and dissolved in ammonia. The resulting solution was boiled until most of the ammonia had been removed and the tyrosine that separated was filtered out. When dried, this weighed 2.4 grams, equal to 1.2 per cent of the gliadin. Recrystallized from boiling water, this gave the following results on analysis:

Carbon and hydrogen: 0.3661 gram substance, dried at 110°, gave 0.7981 gram CO_2 and 0.2160 gram H_2O.

Calculated for $C_9H_{11}O_3N$, C 59.62, H 6.13 p. ct.; found, C 59.45, H 6.56 p. ct.

Tyrosine separated from our hydrolysis solutions of gliadin with very great difficulty. Two other attempts to determine its proportion, which were made by hydrolyzing with sulphuric acid, gave lower results, and the

[1] Cf. Hopkins & Cole, Journal of Physiology, 1901, XXVII, p. 418.

solutions from which the tyrosine separated still continued to give a strong Millon's reaction. Kutscher[1] found 2.09 per cent of tyrosine in gliadin, and Abderhalden & Samuely[2] found 2.37 per cent.

HISTIDINE.

Fifty grams of gliadin, equal to 43.97 grams dried at 110°, were hydrolyzed according to the directions of Kossel & Kutscher[3] and the determination of the bases carried out according to the method of Kossel & Patten.[4]

The solution of the histidine was made up to 500 cc. and nitrogen determined in 100 cc. of it.

100 cc. of solution gave ammonia = 1.37 cc. HCl (1 cc. HCl = 0.01 gram N) = 0.0137 gram N = 0.0685 gram N in 500 cc. = 0.2524 gram histidine = 0.58 p. ct. of the gliadin.

The identity of this histidine was not established, as the quantity was too small to permit the preparation of a satisfactory product.

ARGININE.

The arginine solution was made up to 500 cc. and nitrogen determined in 50 cc. of it.

50 cc. of solution gave ammonia = 4.15 cc. HCl (1 cc. HCl = 0.01 gram N) = 0.0415 gram N or 0.415 gram in 500 cc. = 1.39 gram arginine or 3.16 p. ct. of the gliadin.

The remaining solution was treated as Kossel & Kutscher direct and the arginine obtained as carbonate. A portion of this carbonate was converted into the picrolonate according to the directions of Steudel.[5] This melted at 226° to 227°, while Steudel gives 225°.

Nitrogen: 0.0832 gram substance, dried at 110°, gave 18.8 cc. moist N_2 at 765 mm. and 25°.

Calculated for $C_6H_{14}O_2N_4 \cdot C_{10}H_8O_5N_4$, N 25.62 p. ct.; found, N 25.40 p. ct.

LYSINE.

The filtrate from the silver precipitate of arginine and histidine was freed from silver, precipitated with phosphotungstic acid, and lysine tested for with picric acid in the usual way, but none was found.

Kossel & Kutscher[6] found, in the three fractions of the alcohol-soluble protein of the wheat kernel which they examined, 1.2, 0.43, and 1.53 per cent of histidine. The writer's determination falls between these. Kutscher,[7] in discussing the individuality of these three fractions, considers the differ-

[1] Kutscher, Zeitschrift für physiologische Chemie, 1903, XXXVIII, p. 111.
[2] Abderhalden & Samuely, ibid., 1905, XLIV, p. 276.
[3] Kossel & Kutscher, ibid., 1900, XXXI, p. 165.
[4] Kossel & Patten, ibid., 1903, XXXVIII, p. 39.
[5] Steudel, ibid., 1902, XXXVII, p. 219.
[6] Kossel & Kutscher, loc. cit.
[7] Kutscher, loc. cit.

ences in the amount of histidine found in them to be within the limits of accuracy of these determinations.

Kossel & Kutscher also found 3.05 per cent of arginine in the fraction which they called gluten-fibrin, 2.75 per cent in their gliadin, and 3.13 per cent in their mucedin. In determining the amount of protein hydrolyzed they calculated the weight from the nitrogen in solution. If, as seems probable, only one alcohol-soluble protein exists in this seed, namely, gliadin, with 17.5 per cent of nitrogen, and that the nitrogen of their solutions belonged to this protein, the proportion of arginine found by them would be, respectively, 3.13, 2.79, and 3.25 per cent, with which 3.16 per cent agrees very closely. The results of this hydrolysis are given in table 17.

TABLE 17.—*Gliadin.*

	P. ct.		*P. ct.*
Glycocoll	0.00	Cystine	0.45
Alanine	2.00	Oxy-proline	....
Amino-valerianic acid	0.21	Lysine	0.00
Leucine	5.61	Histidine	0.58
α-proline	7.06	Arginine	3.16
Phenylalanine	2.35	Ammonia	5.11
Aspartic acid	0.58	Tryptophane	present
Glutaminic acid	37.33		
Serine	0.13		65.78
Tyrosine	1.20		

PROTEIN INSOLUBLE IN WATER, SALINE SOLUTIONS, AND ALCOHOL—GLUTENIN.

As already stated, extraction with the above-named solvents, applied successively, removed but a part of the total protein contained in the wheat kernel, that remaining being soluble only in dilute acids and alkalis. The following extractions were next made to determine the nature of this body.

PROTEIN EXTRACTED BY DILUTE ALKALINE SOLUTIONS AFTER EXTRACTING THE FLOUR WITH 10 PER CENT SODIUM-CHLORIDE BRINE AND THEN WITH DILUTE ALCOHOL.

After completely extracting from 4000 grams "straight flour" all the protein soluble in 10 per cent sodium chloride solution, the residue was freed wholly from protein soluble in cold alcohol of 0.90 sp. gr. The resulting residue was then extracted twice with a large quantity of 0.1 per cent potassium hydroxide solution. After standing 3 days at a temperature of 5° with frequent stirring, this extract was filtered off and allowed to stand in a cold room until the greater part of the fine starch and other impurities which had escaped filtration had settled. The solution, which was still turbid, was decanted from the sediment and neutralized as exactly as possible with 0.2 per cent hydrochloric acid, thereby producing a precipi-

tate which settled rapidly, leaving the filtrate milky in appearance. This precipitate, after decanting the solution, was dissolved in 0.2 per cent potassium-hydroxide water and set aside to deposit the suspended impurities. After decantation from the sediment which resulted, the solution was filtered, but almost nothing was thus removed. It was then neutralized with 0.2 per cent hydrochloric acid and the precipitate produced washed by decantation, first with water and then with dilute alcohol, absolute alcohol, and ether. No attempt was made in this case to obtain the whole of the protein soluble in alkali, as the difficulties presented by slow and imperfect filtration rendered this impossible. The substance thus obtained formed a brownish, horny mass, which weighed 13 grams. This preparation, 89, when dried at 110°, on analysis gave the results shown in the table at the bottom of this page.

Subsequent preparations of this body led to the idea that it was far from pure, this supposition being supported by the fact that the final solution from which it had been precipitated was turbid. The remainder of the preparation was then dissolved as completely as possible in 0.2 per cent potassium-hydroxide solution, and by repeated filtration through very dense filter-paper obtained perfectly clear. A considerable insoluble residue remained, which appeared to consist largely of the coagulated form of this protein. This residue was washed by decantation with water, alcohol, and ether. It was found to contain but 13.68 per cent of nitrogen, showing that the preparation, 89, contained much non-nitrogenous matter. The filtration of the dissolved protein proceeded very slowly, so that it was conducted at a temperature near 0°. A portion of the filtrate first obtained was removed, precipitated by 0.2 per cent hydrochloric acid, washed with water, alcohol, and ether, and the preparation, 90, analyzed with the following results. A second portion similarly yielded preparation 91.

Preparations 89, 90, and 91.

	Preparation 89.		Preparation 90.				Preparation 91.	
	I.	Ash-free.	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.47	52.91	51.10	.....	51.10	52.29	.....	.....
Hydrogen .	6.75	6.81	6.46	.....	6.46	6.61	.....	.....
Nitrogen...	15.51	15.65	17.01	.....	17.01	17.41	17.17	17.33
Sulphur....	0.86	0.86	0.91	0.92	0.92	0.94	.....	.....
Oxygen....	.....	23.77	.....	.....	.....	22.75	.....	.....
Ash.	0.88	.....	2.28	.....	.....	.....	0.97	.....
	.....	100.00	.....	.....	.....	100.00	.....	.....

These results show that it is absolutely necessary to filter the alkaline solution of this body *perfectly clear* before the final precipitation, since otherwise a considerable amount of non-nitrogenous matter will be precipitated with it. Another extraction was made by treating 200 grams of "patent flour" from spring wheat with 10 per cent sodium-chloride solution added in small quantities so as to make a dough. This dough was then washed with 10 per cent sodium-chloride brine until nearly all the starch was removed and a gluten obtained similar in all respects to that resulting from treating the flour with water. This gluten was then chopped fine, thoroughly extracted with alcohol of sp. gr. 0.90, and then dissolved at 20° in 0.1 per cent potassium-hydroxide water. The resulting solution was filtered, but, as only a part of the impurities was thus removed, the filtrate was placed in an ice-box in shallow dishes and allowed to deposit a considerable part of the suspended matter. The decanted solution, which was only very slightly turbid, was then precipitated by neutralizing with 0.2 per cent hydrochloric acid, and the precipitate washed by decantation with water, extracted thoroughly with dilute alcohol, digested with absolute alcohol and then with ether. This preparation, 92, was a snow-white, light, porous mass, easily reduced to a powder. It had the following composition :

Preparation 92.

	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct*	*P. ct.*	*P. ct.*
Carbon	.....	52.18	.....	52.50
Hydrogen	.....	6.90	.....	6.94
Nitrogen	17.11	16.90	17.01	17.22
Sulphur	.....	1.00	.....	1.00
Oxygen	.....	.....	.....	22.34
Ash	.....	0.63	.....	.....
	.....	.....	.....	100.00

PROTEIN EXTRACTED BY DILUTE ALKALINE SOLUTIONS FROM GLUTEN AFTER EXTRACTING THE LATTER WITH DILUTE ALCOHOL.

2000 grams of "straight flour" from spring wheat were made into a dough with distilled water, and this was washed with river-water until the gluten was freed as completely as possible from starch. This gluten was then extracted with 75 per cent alcohol as long as anything was removed. The insoluble residue was dissolved in 0.15 per cent potassium-hydroxide water and the resulting solution allowed to stand in a cold room for 48 hours. The solution was thus freed from but a part of the suspended matter. After decanting from the sediment, the solution was neutralized with dilute hydrochloric acid, the precipitate produced, washed several times by decantation

with water, thoroughly extracted with alcohol of 0.90 sp. gr., then with stronger alcohol, and finally with absolute alcohol and with ether.

The precipitate was again dissolved in 0.1 per cent potassium-hydroxide solution and allowed to stand over night. It was then filtered, and a part of the clear filtrate first obtained was precipitated by neutralization with 0.2 per cent hydrochloric acid. This precipitate was washed with water, alcohol, absolute alcohol, and ether, yielding preparation 93. A part of this preparation, 93, was redissolved in 0.2 per cent potassium-hydroxide water and found to contain a considerable amount of substance which had become insoluble in consequence of drying. This insoluble portion was filtered off, washed with water, alcohol, and ether, and gave preparation 94. The filtrate from this substance was precipitated with 0.2 per cent hydrochloric acid, and the precipitate filtered off and washed with water, alcohol, and ether. Through an accident this preparation dried on the filter and could not be removed from the paper. It was then again dissolved in dilute potassium-hydroxide water and treated exactly as before, yielding preparation 95. The following analyses show the composition of these three preparations:

Preparations 93, 94, and 95.

	I.	II.	Average.	Ash-free.
Preparation 93:	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	51.59	.....	51.59	52.32
Hydrogen	6.72	.....	6.72	6.82
Nitrogen	17.34	17.39	17.37	17.61
Sulphur, Oxygen	.....	.....	.....	23.25
Ash	1.40	.....	.....	.....
	.....	.....	.....	100.00
Preparation 94:				
Carbon	50.79	.....	50.79	52.87
Hydrogen	6.62	.....	6.62	6.88
Nitrogen	16.38	16.38	16.38	17.05
Sulphur, Oxygen	.....	.....	.....	23.20
Ash	3.94	.....	3.94	.....
	.....	.....	.....	100.00
Preparation 95:				
Carbon	51.50	51.37	51.44	52.62
Hydrogen	6.70	6.57	6.64	6.80
Nitrogen	16.70	.....	16.70	17.08
Sulphur, Oxygen	.....	.....	.....	23.50
Ash	2.25	.....	.....	.....
	.....	.....	.....	100.00

Another lot of gluten made from 1000 grams of "straight flour" was treated in exactly the same way as that last described. This gluten was thoroughly extracted with alcohol of 0.90 sp. gr. and the residue dissolved in about 500 cc. of 0.2 per cent potassium-hydroxide water. After standing over night, a very turbid liquid was decanted from the sediment which had formed and treated with very dilute acetic acid added to slightly acid reaction. The precipitate produced was washed with water, alcohol, and ether and dissolved again in 0.2 per cent potassium-hydroxide water. The resulting solution, filtered perfectly clear, was precipitated with 0.2 per cent hydrochloric acid, washed by decantation with water, then with dilute alcohol increased gradually in strength up to absolute alcohol, and finally with ether. When dried over sulphuric acid, a pure white, light mass was obtained. This preparation, 96, was analyzed with the results shown in the table below.

Again, gluten was prepared in the usual manner and extracted with alcohol until everything soluble in that reagent was removed. The residue was then dissolved in 0.2 per cent potassium-hydroxide water and, in order to carry the operation to an end as rapidly as possible, the solution was at once thrown into a filter. As soon as the filtrate ceased to pass through turbid it was returned to the funnel and the filtration continued in an ice-chest. A considerable portion of the solution was obtained perfectly clear after 20 hours. This was then precipitated with 0.2 per cent hydrochloric acid and treated in the usual manner, giving preparation 97.

Preparations 96 and 97.

	Preparation 96.				Preparation 97.	
	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.07	52.23	52.15	52.54	52.07	52.38
Hydrogen ..	6.71	6.88	6.80	6.85	6.77	6.81
Nitrogen....	17.23	17.42	17.33	17.46	17.49	17.59
Sulphur	1.07	.....	1.07	1.07	1.24	1.24
Oxygen.....	.....	.....	.....	22.08	.....	21.98
Ash	0.74	0.76	0.75	.....	0.61	.....
	.....	.....	.....	100.00	.....	100.00

In order to determine whether a loss of nitrogen occurred through prolonged contact with the alkaline solution, the following experiment was tried, in which the conditions under which preparation 97 was made were repeated and preparation 98 obtained. The rest of the alkaline solution was kept in the ice-box for 3 days longer, during which time the ice melted and the

temperature rose to 20°. The preparation, 99, obtained by neutralizing the solution with hydrochloric acid contained the same amount of nitrogen as preparation 98, from which it is evident that prolonged solution in dilute potassium hydroxide solution caused no loss of nitrogen.

Preparations 98, 99, and 100.

	Preparation 98.		Preparation 99.		Preparation 100.			
	I.	Ash-free.	I.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Nitrogen ...	17.32	17.53	17.50	17.53	17.04	16.93	16.99	17.20
Ash	1.23	.....	0.16	.....	1.22	.....	.....	.....

Another preparation of this substance (preparation 100) was made by completely extracting 200 grams of "patent flour" (from spring wheat) with large quantities of alcohol of 0.90 sp. gr. The flour was then washed with absolute alcohol and air-dried. The dry material was then powdered and made into a dough with distilled water which had considerable coherence, showing that the protein insoluble in alcohol played an important part in its formation. This dough was then washed on a fine hair-sieve under a stream of running water, but no coherent gluten resulted. The washings were then allowed to settle, and the sediment, after decanting the solution, was treated with 0.2 per cent potassium-hydroxide water. The solution so obtained, after standing over night, was decanted from the sediment and precipitated with 0.2 per cent hydrochloric acid and the separated protein allowed to settle. The solution was then decanted, the precipitate dissolved in 0.2 per cent potassium-hydroxide water, and filtered clear in the ice-box. The filtered solution was then precipitated and the separated substance treated in the usual manner. This preparation, 100, contained nitrogen, as shown in the above table.

In order to learn whether any change in the protein occurred in consequence of contact with aqueous solutions before extracting with potassium-hydroxide water, the following experiment was made:

1000 grams of "straight flour" from spring wheat were repeatedly extracted with alcohol of 0.90 sp. gr., and after removing everything soluble in that liquid the alcohol was squeezed out as completely as possible in a screw-press and the residue extracted with 0.2 per cent potassium-hydroxide water. It was found impossible to separate the solution from the undissolved portion of the meal, either by filtration or subsidence, on account of the presence of gummy matter. An equal volume of alcohol of sp. gr. 0.820

was therefore added, and on long standing the insoluble substance gradually settled, leaving a comparatively clear, yellow solution. This was then siphoned off and filtered. On account of its gummy character it filtered very slowly. The clear solution finally obtained was then precipitated with 0.2 per cent hydrochloric acid, the precipitate filtered off and dissolved in 0.2 per cent potassium-hydroxide water. The solution so obtained was filtered clear and precipitated with 0.2 per cent hydrochloric acid, the resulting precipitate washed by decantation with water, alcohol—at first dilute, then gradually increased to absolute—and finally with ether. Preparation 101 was thus obtained, having the composition shown in the following table:

Preparations 101 and 102.

	Preparation 101.		Preparation 102.			
	I.	Ash-free.	I.	II.	Average.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon.....	52.14	52.19	51.59	51.67	51.63	52.19
Hydrogen..	6.91	6.92	6.85	6.87	6.86	6.93
Nitrogen...	17.54	17.56	17.21	17.32	17.27	17.45
Sulphur....	}	23.33	{ 0.89	.....	0.89	0.90
Oxygen.....	}		{	.....	.....	23.43
Ash	0.10	.....	1.07	.....	.....	.....
	.....	100.00	.....	.....	.....	100.00

This analysis shows that the protein extracted by potassium hydroxide water from the flour which has not been in contact with water has the same composition as that obtained in the other experiments.

PROTEIN EXTRACTED BY DILUTE ALKALINE SOLUTIONS AFTER COMPLETE EXTRACTION WITH DILUTE ALCOHOL OF GLUTEN FROM WHOLE-WHEAT FLOUR.

1000 grams of flour made by grinding the *entire* kernel of spring wheat were made into a dough, washed with water till free from starch, and the gluten obtained extracted thoroughly with dilute alcohol. The residue was then dissolved in 0.2 per cent potassium-hydroxide water, and after the resulting solution had stood for some time it was decanted from the sediment and precipitated by 0.2 per cent hydrochloric acid. The precipitate was washed by decantation with water, thoroughly extracted with dilute alcohol and then with absolute alcohol, and finally with ether, and then redissolved in 0.2 per cent potassium-hydroxide water.

The solution, after filtering perfectly clear, was precipitated and the precipitate treated in the same manner as all the preceding preparations. When

analyzed, preparation 102 was found to have the composition shown in the table on page 88.

The filtrate from this preparation, as well as all the others previously described, contained a small amount of protein matter. This was then treated with a solution of copper sulphate and the small precipitate thereby produced filtered off, washed with water, alcohol, and ether (preparation 103), and found to contain the following amount of nitrogen :

Preparation 103.

	I.	Ash-free.
	P. ct.	*P. ct.*
Nitrogen	13.28	17.45
Ash	23.88	.....

In the same way as 102 a preparation of this protein was made from flour from the *entire* kernel of winter wheat. The composition of this preparation is shown by the figures in the following table for preparation 104.

Preparations 104 and 105.

	Preparation 104.				Preparation 105.	
	I.	II.	Average.	Ash-free.	I.	Ash-free.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	51.71	.....	51.71	52.03	51.17	52.44
Hydrogen ..	6.79	.....	6.79	6.83	6.70	6.86
Nitrogen....	17.32	17.44	17.38	17.48	17.35	17.81
Sulphur .... }	.....	.....	.....	23.66	.....	22.89
Oxygen..... }						
Ash	0.62	.....	0.62	.....	2.62	.....
	.....	.....	.....	100.00	.....	100.00

A second portion of the same solution from which preparation 104 had been precipitated was obtained 2 days later. This was then treated in the same way and yielded preparation 105, which was found to have the composition shown in the table above.

If preparations 89 and 92 are omitted on account of being obtained from unfiltered solutions, 94 as an altered and insoluble product, and 95 as having been subjected to an exceedingly prolonged action of an alkaline solution, then will be had, in table 18, the analyses which, in the opinion of the writer, most nearly represent the true composition of this protein.

TABLE 18.—*Protein of the wheat kernel soluble only in dilute acids and alkalis.*

	90.	91.	93.	96.	97.	98.	99.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.29	.....	52.32	52.54	52.38	.....	.....
Hydrogen	6.61	.....	6.82	6.85	6.81	.....	.....
Nitrogen	17.41	17.33	17.61	17.46	17.59	17.53	17.53
Sulphur	.94	.....	23.25	1.07	1.24	.....	.....
Oxygen	22.75	.....		22.08	21.98	.....	.....
	100.00	.....	100.00	100.00	100.00	.....	.....

	100.	101.	102.	103.	104.	105.	Average.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	.....	52.19	52.19	.....	52.03	52.44	52.34
Hydrogen	.....	6.92	6.93	.....	6.83	6.86	6.83
Nitrogen	17.20	17.56	17.45	17.45	17.48	17.81	17.49
Sulphur	.....	23.33	23.43	.....	23.66	22.89	1.08
Oxygen	.....						22.26
	.....	100.00	100.00	.....	100.00	100.00	100.00

There can be no doubt that the earlier analyses of this protein, with the exception of those made by Ritthausen and Chittenden & Smith, are incorrect, for the substance analyzed contained a large part of the impurities of the gluten, since after extraction with alcohol the residue was not dissolved in any solvent, and the starch, bran, etc., contained in it filtered off. Ritthausen's figures are the average of two analyses of preparations obtained from solutions in 0.2 per cent potassium-hydroxide water which had been filtered, one perfectly clear, giving a product containing 17.21 per cent N, the other very nearly clear and containing 17.08 per cent N.

It is evident that the alkaline solution of the gluten that has previously been extracted with cold dilute alcohol contains something which requires filtration through very dense paper to effect its separation. Wheat gluten contains a considerable amount of phytocholesterin and lecithin, together with fat, and it is probable that all these bodies are held suspended in the solution of the dissolved protein as an emulsion, and can only be removed by precipitating the first solution in potassium-hydroxide water, extracting the precipitate with alcohol and ether, redissolving, and filtering the resulting solution perfectly clear. The first alkaline solution before precipitating and extracting with alcohol and ether can not be filtered, as it either passes through turbid or does not run through at all.

On the ground of priority and the fact that the relations to animal proteins which gave rise to the various names subsequently applied to this body have

been proved to have no foundation, it would be desirable to return to Taddei's original name and in future call this protein zymon. Unfortunately this name is derived from the Greek word ζύμη, a ferment, and, as the results of this investigation show that the supposed ferment-changes do not occur in the formation of gluten, this name is undesirable. As this protein is especially characteristic of gluten, it seems appropriate to call it *glutenin*, a name suggested by Prof. S. W. Johnson.

Hydrolysis of Glutenin.

The large quantity of glutenin which was required for the quantitative determination of its decomposition products was prepared from the residue of the wheat gluten after extracting the gliadin with alcohol. This residue was dried at room temperature and then ground to a powder, which was extracted first with absolute alcohol and then with ether as long as either solvent removed anything from it. The alcohol was then removed at room temperature and the residual powder treated with just enough 0.2 per cent solution of potassium hydroxide to dissolve it. The resulting turbid solution was then filtered perfectly clear and neutralized with very dilute hydrochloric acid. The precipitate produced was extracted with 70 per cent alcohol as long as any gliadin was removed, then thoroughly dehydrated with absolute alcohol, and dried over sulphuric acid.

940 grams, equal to 839.32 grams of glutenin, ash and water free, were hydrolyzed by heating for several hours on a water-bath with a mixture of 950 cc. of concentrated hydrochloric acid and 950 cc. of water. After standing over night, the solution was boiled on an oil-bath for 9 hours, and then saturated with hydrochloric acid gas. By the same treatment as that applied to gliadin (p. 71) 202.73 grams glutaminic acid hydrochloride, equal to 162.40 grams of the free acid, were obtained. Recrystallized once from concentrated hydrochloric acid, this melted at 198°.

Chlorine: 0.5386 gram substance gave 0.4211 gram AgCl.
Nitrogen: 0.5911 gram substance gave $NH_3 = 4.53$ cc. HCl (1 cc. HCl = 0.01 gram N).
Calculated for $C_5H_{10}O_4NCl$, Cl 19.35, N 7.65 p. ct.; found, Cl 19.33, N 7.66 p. ct.

The united filtrates and washings were concentrated to a sirup on a water-bath under reduced pressure and the hydrochlorides of the amino-acids esterified three times, as in the case of gliadin. The hydrochlorides of the esters were neutralized and the free esters shaken out with ether, as for gliadin. After drying the ether solution of the esters with potassium carbonate, it was kept 2 days over anhydrous sodium sulphate. The ether was then removed by distillation from a water-bath at atmospheric pressure and the esters distilled, with the results shown in the table on page 92 for distillation A. The undistilled residue weighed 211.5 grams.

Distillations A and B.

Fraction.	Distillation A. Temperature of bath up to—	Pressure.	Weight.	Fraction.	Distillation B. Temperature of bath up to—	Pressure.	Weight.
	°	*mm.*	*Grams.*		°	*mm.*	*Grams.*
I.....	65	12.0	61.00	I.....	65	12.0	28.74
II.....	100	12.0	43.74	II.....	88	12.0	24.27
III *a*.....	100	4.0	45.94	III *a*.....	100	10.0	19.18
III *b*.....	110	1.5	33.01	III *b*.....	120	0.8	23.23
IV.....	155	1.5	43.32	IV.....	180	0.8	23.92
V.....	200	0.8	32.56				
	.....	.....	259.57		.....	.....	119.34

The residue, from which the esters had been removed by ether, was subjected to two or more esterifications, as in the case of gliadin, and the esters distilled. (See distillation B in the table above.) The undistilled residue weighed 93 grams.

The different fractions from the two distillations were worked up as follows:

Fraction.	Temperature of bath up to —	Pressure.	Weight.
	°	*mm.*	*Grams.*
I A.....	65	12	61.00
I B.....	65	12	28.74

This was saponified at once by evaporating to a sirup with concentrated hydrochloric acid and the residue dissolved in alcohol and esterified with dry hydrochloric acid gas. The glycocoll ester hydrochloride which separated weighed 6.68 grams. Recrystallized from alcohol, this melted at 144°.

Carbon and hydrogen: 0.3179 gram substance gave 0.3984 gram CO_2 and 0.2151 gram H_2O.

Calculated for $C_4H_{10}O_2NCl$, C 34.39, H 7.23 p. ct.; found, C 34.18, H 7.52 p. ct.

The filtrate from the glycocoll ester hydrochloride was saponified by evaporating on the water-bath with hydrochloric acid, the latter removed with silver sulphate, and the solution freed from sulphuric acid with an

equivalent quantity of barium hydroxide. By fractional crystallization this solution yielded 11.83 grams of alanine, which melted at about 290°.

Carbon and hydrogen: 0.2701 gram substance, dried at 110°, gave 0.4001 gram CO_2 and 0.1946 gram H_2O.

Nitrogen: 0.3460 gram substance, dried at 110°, gave $NH_3 = 5.44$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_3H_7O_2N$, C 40.40, H 7.93, N 15.75 p. ct.; found, C 40.40, H 8.01, N 15.73 p. ct.

Fraction.	Temperature of bath up to—	Pressure.	Weight.
	°	*mm.*	*Grams.*
II A.....	100	12	43.74
II B.....	88	10	24.27

The united esters were saponified by boiling with 10 parts of water for 5 hours, when their solution reacted neutral to litmus. This solution was evaporated to dryness under reduced pressure and boiled up with absolute alcohol, whereby 0.5 gram substance was dissolved. By repeated fractional crystallization of the substance insoluble in alcohol, 3.5 grams leucine were obtained. The isolated leucine decomposed at about 298°.

Carbon and hydrogen: 0.2408 gram substance, dried at 110°, gave 0.4852 gram CO_2 and 0.2169 gram H_2O.

Calculated for $C_6H_{13}O_2N$, C 54.89, H 10.01 p. ct.; found, C 54.95, H 10.01 p. ct.

After adding to the filtrate from this leucine the most soluble portion of fraction III, and further fractioning, 25.76 grams of alanine and 3.84 grams of glycocoll were obtained. The latter was isolated as the hydrochloride of the ester, which melted at 144°. The alanine was racemized and the α-naphthyl-hydantoic acid prepared according to the directions of Neuberg & Manasse.[1] When recrystallized from dilute alcohol, this melted at 197°.

Carbon and hydrogen: 0.2527 gram substance, dried at 90°, gave 0.5998 gram CO_2 and 0.1280 gram H_2O.

Nitrogen: 0.3515 gram substance, dried at 90°, gave $NH_3 = 3.74$ cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_{14}H_{14}O_3N_2$, C 65.06, H 5.48, N 10.87 p. ct.; found, C 64.74, H 5.61, N 10.61 p. ct.

[1] Neuberg & Manasse, Berichte der deutschen chemischen Gesellschaft, 1905, XXXVIII, p. 2359.

Fraction.	Temperature of bath up to—	Pressure.	Weight.
	°	*mm.*	*Grams.*
III { A.....	110	1.5	78.95
III { B.....	120	0.8	42.41

These esters were saponified by boiling with 6 parts of water for 5 hours, when their solution reacted neutral to litmus. After evaporating to dryness under reduced pressure, the dried residue was extracted with boiling absolute alcohol. The part insoluble in alcohol, by a systematic fractional crystallization, gave 32.42 grams of leucine, which decomposed at about 298°.

Carbon and hydrogen: 0.4255 gram substance, dried at 110°, gave 0.8535 gram CO_2 and 0.3926 gram H_2O.

Nitrogen: 0.1488 gram substance gave NH_3 = 1.60 cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_6H_{13}O_2N$, C 54.89, H 10.01, N 10.70 p. ct.; found, C 54.71, H 10.25, N 10.77 p. ct.

The filtrate from this leucine yielded 2 grams of substance, which had the composition and properties of amino-valerianic acid.

Carbon and hydrogen: 0.2216 gram substance gave 0.4144 gram CO_2 and 0.1967 gram H_2O.

Calculated for $C_5H_{11}O_2N$, C 51.22, H 9.48 p. ct.; found, C 51.00, H 9.86 p. ct.

Specific rotation.—Dissolved in 20 per cent hydrochloric acid,

$$(\alpha)\ \frac{20°}{D} = +25.63°$$

A similar preparation from gliadin gave +25.79°. E. Fischer & Dörpinghaus[1] found +25.9° for a preparation from horn, and E. Schulze & Winterstein[2] found +28.2° and +27.9° for a preparation from lupine seedlings.

The solution used for determining the specific rotation was freed from hydrochloric acid with silver sulphate, and the amino-acids racemized by heating with an excess of barium hydroxide in an autoclave at 175° for 19 hours. After removing the barium quantitatively with sulphuric acid, the substance was coupled with α-naphthylisocyanate. The hydantoic acid melted constantly, on repeated recrystallization from dilute alcohol, at 183° to

[1] Fischer, E., & Dörpinghaus, Zeitschrift für physiologische Chemie, 1902, XXXVI, p. 462.

[2] Schulze, E., & Winterstein, ibid., 1902, XXXV, p. 300.

184°. Heated side by side with the corresponding substance obtained from gliadin, the hydantoic acid from glutenin melted at 2° higher.

Carbon and hydrogen: 0.3277 gram substance, dried at 80°, gave 0.8067 gram CO_2 and 0.1891 gram H_2O.

Calculated for $C_{16}H_{18}O_3N_2$, C 67.07, H 6.35 p. ct.; found, C 67.14, H 6.42 p. ct.

From the filtrate from the amino-valerianic acid there was further obtained 1.41 grams of alanine. The alcoholic solution which contained the proline was evaporated to dryness under reduced pressure and the dried residue again treated with boiling absolute alcohol. Even after several repetitions of this process no substance insoluble in alcohol could be obtained. The alcohol-soluble substance, when dried, weighed 35.54 grams. A copper salt was prepared from this in the usual manner, and its solution evaporated to dryness under reduced pressure. The dried residue was extracted with boiling absolute alcohol in order to remove the *l*-proline copper. The residue insoluble in alcohol, when recrystallized from water, gave 15.53 grams of racemic proline copper, equivalent to 10.9 grams of proline.

Water: 0.3618 gram substance lost at 110° 0.0399 gram of H_2O.

Copper: 0.3176 gram substance, dried at 110°, gave 0.0856 gram CuO.

Calculated for $C_{10}H_{16}O_4N_2\,Cu \cdot 2H_2O$, H_2O 11.00 p. ct.; found, H_2O 11.03 p. ct.

Calculated for $C_{10}H_{16}O_4N_2\,Cu$, Cu 21.79 p. ct.; found, Cu 21.54 p. ct.

The alcoholic solution of the *l*-proline copper salt was evaporated to dryness, the copper removed, and the proline identified as the phenylhydantoin which was prepared according to the directions of Fischer.[1] The substance thus prepared was at once pure and melted at 143°.

Carbon and hydrogen: 0.2822 gram substance, dried in vacuo over H_2SO_4, gave 0.6866 gram CO_2 and 0.1494 gram H_2O.

Nitrogen: 0.1025 gram substance gave NH_3 = 1.33 cc. HCl (1 cc. HCl = 0.01 gram N).

Calculated for $C_{12}H_{12}O_2N_2$, C 66.60, H 5.61, N 12.99 p. ct.; found, C 66.36, H 5.88, N 12.98 p. ct.

{ Fraction IV, A. Temperature of bath, up to 155°. }
{ Pressure, 1.5 mm. Weight, 43.32 grams. }

This fraction was shaken out with ether in the usual way and the ether allowed to evaporate spontaneously. No evidence of the presence of phenylalanine was obtained. The residue of ester was saponified with concentrated hydrochloric acid and the hydrochloride decomposed with ammonia. The free acid crystallized in the characteristic form of leucine and decomposed at 298°. There were obtained 13.99 grams of leucine.

Carbon and hydrogen: 0.2906 gram substance, dried at 110°, gave 0.5825 gram CO_2 and 0.2690 gram H_2O.

Calculated for $C_6H_{13}O_2N$, C 54.89, H 10.01 p. ct.; found, C 54.67, H 10.28 p. ct.

[1] Fischer, E., Zeitschrift für physiologische Chemie, 1901, XXXIII, p. 151.

The aqueous layer was saponified by heating on a water-bath with an excess of barium hydroxide for 5 hours. The barium aspartate, which separated in considerable quantity on standing, was united with that obtained from fraction V and treated as will be described later.

From the filtrate from the barium aspartate no definite substance could be obtained. It appeared to contain serine, but none could be isolated, even by the use of β-naphthalene-sulphone-chloride.

Fraction.	Temperature of bath up to—	Pressure.	Weight.
	°	*mm.*	*Grams.*
V, A.....	200	0.8	32.56
IV, B.....	180	0.8	23.92

These esters were shaken out with ether, and the substance extracted was saponified with hydrochloric acid. The hydrochloride thus obtained, which weighed 20.21 grams, equal to 16.55 grams free phenylalanine, was converted into the free acid with ammonia and then into the copper salt by boiling its solution with copper hydroxide.[1]

Copper: 0.2099 gram substance, dried at 110°, gave 0.0425 gram CuO.
Nitrogen: 0.2283 gram substance gave NH_3 = 1.63 cc. HCl (1 cc. HCl = 0.01 gram N). Calculated for $C_{18}H_{20}O_4N_2Cu$, Cu 16.23, N 7.17 p. ct.; found, Cu 16.18, N 7.14 p. ct.

The free phenylalanine isolated from this copper salt melted at 263° to 265°.

Nitrogen: 0.1085 gram substance gave NH_3 = 0.92 cc. HCl (1 cc. HCl = 0.01 gram N). Calculated for $C_9H_{11}O_2N$, N 8.50 p. ct.; found, N 8.48 p. ct.

The aqueous layer was saponified by heating for 5 hours with an excess of barium hydroxide on the water-bath. The barium aspartate, which separated on standing, was united with that previously obtained from fraction IV, A, decomposed with an equivalent amount of sulphuric acid, and 7.12 grams of free aspartic acid were obtained from the solution.

Carbon and hydrogen: 0.3293 gram substance, dried at 110°, gave 0.4335 gram CO_2 and 0.1625 gram H_2O.
Nitrogen: 0.2997 gram substance gave NH_3 = 3.17 cc. HCl (1 cc. HCl = 0.01 gram N). Calculated for $C_4H_7O_4N$, C 36.05, H 5.31, N 10.55 p. ct.; found, C 35.90, H 5.48, N 10.58 p. ct.

The filtrate from barium aspartate was freed from barium, concentrated under reduced pressure, and saturated with hydrochloric acid. On long standing on ice a trace of phenylalanine hydrochloride separated, but no glutaminic acid hydrochloride could be obtained. After removing the

[1] Cf. Schulze & Winterstein, Zeitschrift für physiologische Chemie, 1902, XXXV, p. 210.

hydrochloric acid with silver sulphate, the solution was boiled with a solution of copper hydroxide and 1.1 grams of copper aspartate were isolated.

Copper: 0.3991 gram substance, air-dried, gave 0.1163 gram CuO.
Nitrogen: 0.2066 gram substance gave NH_3 = 1.06 cc. HCl (1 cc. HCl = 0.01 gram N).
Calculated for $C_4H_5O_4N$, Cu · 4½ H_2O,[1] Cu 23.06, N 5.09 p. ct.; found, Cu 23.27, N 5.13 p. ct.

The filtrate from this copper aspartate, when freed from copper by hydrogen sulphide and concentrated to small volume, yielded, by fractional crystallization, 4.35 grams of nearly pure serine.

Carbon and hydrogen: 0.2847 gram substance gave 0.3559 gram CO_2 and 0.1751 gram H_2O.
Calculated for $C_3H_7O_3N$, C 34.29, H 6.67 p. ct.; found, C 34.09, H 6.83 p. ct.

This substance browned at about 213° and decomposed to a brownish mass at about 243°.

Residue from Distillation.

The residues which remained after distilling off the esters weighed 304.5 grams. These were dissolved in hot alcohol, and from their united solutions 3.16 grams of substance separated on cooling. The filtrate from this substance was evaporated under reduced pressure, the residue dissolved in water and saponified by heating with an excess of barium hydroxide for 9 hours. After removing the barium quantitatively, the solution was concentrated and saturated with hydrochloric acid. On standing for some time on ice, 38.25 grams of glutaminic acid hydrochloride, which melted at 198°, were obtained. This was equivalent to 30.64 grams of free glutaminic acid, and, with that previously isolated, made a total of 193.04 grams, or 23 per cent of the glutenin. The glutaminic acid hydrochloride was decomposed with an equivalent quantity of potassium hydroxide, and the free glutaminic acid was recrystallized from water. It melted at 202° to 203° with effervescence.

Carbon and hydrogen: 0.3504 gram substance, dried at 110°, gave 0.5275 gram CO_2 and 0.2003 gram H_2O.
Calculated for $C_5H_9O_4N$, C 40.82, H 6.12 p. ct.; found, C 41.06, H 6.35 p. ct.

Residue after Esterification.

The residue which remained after the third esterification and extraction of the esters was treated in the same way as the corresponding residue from gliadin (p. 77), and the solution, freed from all mineral salts and bases precipitable by phosphotungstic acid, was concentrated under reduced pressure to a small volume and then left for some time over sulphuric acid.

[1] Ritthausen, Die Eiweisskörper, etc., Bonn, 1872, p. 219.

After removing a little tyrosine that first separated, 1.87 grams of serine crystallized out, which, when recrystallized from water, browned at about 210° and decomposed at 240°.

Carbon and hydrogen: 0.2308 gram substance, dried at 110°, gave 0.2894 gram CO_2 and 0.1407 gram H_2O.
Calculated for $C_3H_7O_3N$, C 34.29, H 6.67 p. ct.; found, C 34.19, H 6.77 p. ct.

This, with the serine obtained from fraction V, gives a total of 6.22 grams of serine isolated. The mother-liquor from the serine contained considerable substance, but no oxy-proline or other definite substance could be obtained from it.

CYSTINE.

300 grams of glutenin were hydrolyzed in the way described for gliadin (p. 80). After evaporating at low pressure to a sirup, neutralizing the remaining excess of acid with sodium hydroxide, and decolorizing the solution with bone-black, a considerable quantity of tyrosine separated out, which, on examination, was found to contain nearly all the cystine that could be detected in the solution. It was therefore dissolved in 5 per cent sulphuric acid and the cystine precipitated with mercuric sulphate. The mercury precipitate was decomposed with hydrogen sulphide, the solution concentrated somewhat, made alkaline with ammonia and then acid with acetic acid, and an equal volume of alcohol added. The cystine, which separated on standing in characteristic hexagonal plates, weighed only 0.17 gram. No more could be obtained. This was dissolved in ammonia and reprecipitated by acetic acid.

Sulphur: 0.0897 gram substance, dried at 110°, gave 0.1730 gram $BaSO_4$.
Calculated for $C_6H_{12}O_4N_2S_2$, S 26.68 p. ct.; found, S 26.53 p. ct.

Although glutenin contains about the same amount of sulphur as gliadin, the amount of cystine obtained from the latter under similar conditions was very much greater. It would seem as if glutenin in fact yields less cystine, though the uncertainties attending the isolation of this substance will not permit of a positive conclusion.

TYROSINE.

250 grams of glutenin were boiled with a mixture of 750 grams sulphuric acid and 1500 grams of water for 12 hours. The solution was freed from sulphuric acid by an equivalent amount of barium hydroxide, and after concentrating to 800 cc. allowed to stand for some time. A considerable quantity of tyrosine separated, which was filtered out, the filtrate boiled with barium carbonate in order to expel ammonia, and then concentrated to one-half its original volume. After cooling, the residue of barium carbonate

and other substances which had separated were extracted with hot dilute ammonia and the filtered extract concentrated and cooled. On standing, a little more tyrosine separated, which was added to that first obtained. No more tyrosine could be isolated from the solution of the hydrolytic decomposition products. All of the tyrosine which had separated was dissolved in 5 per cent sulphuric acid, and phosphotungstic acid was added to the solution. Only a small precipitate resulted. After removing the phosphotungstic acid with barium hydroxide, the solution was concentrated strongly and allowed to cool. After standing for some time, 9.62 grams of tyrosine were obtained, which is equal to 4.25 per cent of the glutenin.

Carbon and hydrogen: 0.2922 gram substance, dried at 110°, gave 0.6370 gram CO_2 and 0.1634 gram H_2O.
Calculated for $C_9H_{11}O_3N$, C 59.62, H 6.13 p. ct.; found, C 59.45, H 6.21 p. ct.

Kutscher[1] found 2.75 per cent of tyrosine in "gluten-casein."

HISTIDINE.

Fifty grams of glutenin, equal to 43.39 grams dried at 110°, were hydrolyzed, and the arginine and histidine separated in the same way as that described for gliadin (p. 81). The solution containing the histidine was made up to 500 cc.

Nitrogen: 100 cc. solution gave $NH_3 = 4.15$ cc. HCl (1 cc. HCl = 0.01 gram N) = 0.0415 gram N = 0.2075 gram in 500 cc. = 0.7645 gram histidine = 1.76 p. ct. of the glutenin.

The amount of histidine in the remaining solution was too small for identification.

ARGININE.

The filtrate from the histidine precipitate yielded 500 cc. of solution containing the arginine, in which was found the following amount of nitrogen:

Nitrogen: 50 cc. solution gave $NH_3 = 6.79$ cc. HCl (1 cc. HCl = 0.01 gram N) = 0.0679 gram N, or 0.679 gram in 500 cc., or 2.107 gram arginine = 4.72 p. ct. of the glutenin.

The remaining solution, treated as Kossel directs, yielded the arginine as carbonate. This was converted into the copper salt, which gave the following results on analysis:

Carbon and hydrogen: 0.2118 gram substance, air-dried, lost 0.0210 gram H_2O at 100°.
Calculated for $C_{12}H_{28}O_{10}N_{10}Cu \cdot 3\,H_2O$, H_2O 9.15 p. ct.; found, H_2O 9.92 p. ct.
Copper: (I) 0.1858 gram substance, dried at 100°, gave 0.0275 gram CuO; (II) 0.1808 gram substance, dried at 100°, gave 0.0267 gram CuO.
Calculated for $C_{12}H_{28}O_{10}N_{10}Cu$, Cu 11.85 p. ct.; found, Cu (I) 11.84, (II) 11.78 p. ct.

[1] Kutscher, Zeitschrift für physiologische Chemie, 1903, XXXVI, p. 114.

Kossel & Kutscher[1] found in three separate determinations of arginine in glutenin 4.50, 4.02, and 4.54 per cent. They base their determinations on the supposition that glutenin contains 16.2 per cent nitrogen. If their results are recalculated to a basis of 17.5 per cent of nitrogen which has been found in this protein,[2] they become 4.82, 4.52, and 4.84 per cent, with which 4.72 per cent agrees very closely.

LYSINE.

The filtrate from the arginine silver was treated as Kossel directs, and after precipitating the lysine with phosphotungstic acid it was converted into the picrate, and 2.33 grams, equivalent to 0.907 gram of free lysine, were obtained. This is equal to 1.92 per cent of the glutenin. Kossel & Kutscher found in the three determinations of this substance which they made in this protein 1.9, 2.29, and 2 per cent, or, recalculating to a basis of 17.5 per cent of nitrogen in this protein, 2.05, 2.15, and 2.40 per cent.

Nitrogen: 0.1225 gram substance, dried at 120°, gave 23.1 cc. moist N_2 at 760 mm. and 29°.

Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3$, N 18.70 p. ct.; found, N 18.76 p. ct.

The results of this hydrolysis are given in table 19.

TABLE 19.—*Glutenin.*

	P. ct.		P. ct.
Glycocoll	0.89	Tyrosine	4.25
Alanine	4.65	Cystine	0.02
Amino-valerianic acid	0.24	Lysine	1.92
Leucine	5.95	Histidine	1.76
α-proline	4.23	Arginine	4.72
Phenylalanine	1.97	Ammonia	4.01
Aspartic acid	0.91	Tryptophane	present
Glutaminic acid	23.42		
Serine	0.74	Total	59.66

THE AMOUNT OF THE VARIOUS PROTEINS CONTAINED IN THE KERNEL OF WHEAT.

1000 grams of fine meal obtained by freshly grinding the entire kernel of spring wheat and a like quantity of similar flour from winter wheat were each extracted with 4000 cc. of 10 per cent sodium-chloride solution, and 2500 cc. of the clear extract were obtained from the spring-wheat flour and 2600 cc. from the winter-wheat flour. As 100 cc. of solution were used for every 25 grams of flour taken, the amount of extract obtained was approximately equal to that from 625 grams of spring-wheat flour and 650 grams of winter-wheat flour. These extracts were then dialyzed until all the chlo-

[1] Kossel & Kutscher, Zeitschrift für physiologische Chemie, 1900, XXXI, p. 165.
[2] Osborne & Voorhees, American Chemical Journal, 1893, XV, 392.

rides were removed, which required 5 days. The precipitated globulin was then filtered from each, washed with distilled water, alcohol, absolute alcohol, and ether, removed from the filter, and dried at 110°. From the spring-wheat extract 3.8398 grams were obtained, equal to 0.624 per cent of the flour, and from the winter wheat 3.9265 grams, equal to 0.625 per cent.

The filtrates from the globulin were then heated to 65° in a water-bath, and after being held at this temperature for some time the coagulum was filtered off and washed with hot water, alcohol, and ether, removed from the paper, and dried at 110°. From the spring wheat 1.9714 grams were obtained, being 0.315 per cent of the flour, and from the winter wheat 1.9614 grams, equal to 0.302 per cent.

The solutions filtered from each of these coagula were next heated just to boiling and the resulting coagulum filtered off, washed thoroughly, and treated as the preceding preparations had been. The spring-wheat extract thus yielded 0.4743 gram, equal to 0.076 per cent; the winter-wheat extract 0.3680 gram, equal to 0.057 per cent.

The two extracts were next concentrated by boiling down over a lamp. They remained clear at first, but when somewhat concentrated the protein began to separate as a skin on the surface of the solution. When reduced to about one-fourth its original volume, the coagulum was filtered off, washed with boiling water, alcohol, and ether, and dried at 110°. The spring wheat thus yielded 0.8737 gram, equal to 0.139 per cent; the winter wheat 0.8721 gram, equal to 0.134 per cent. The filtrates from these two preparations were evaporated very nearly to dryness on water-baths. On cooling, much substance separated, which, when treated with hot water, dissolved again. The insoluble coagulum was filtered from each, washed, and dried in the usual manner. The spring wheat gave 0.8149 gram of substance, being 0.130 per cent of the flour; the winter wheat 0.5795 gram, being 0.089 per cent. The total amount of protein coagulating on concentration was therefore 0.269 per cent for the spring wheat and 0.223 per cent for the winter wheat.

The filtrates from these second coagula were then again evaporated to a sirup, and as no more insoluble matter separated they were each precipitated by pouring into strong alcohol. Large precipitates resulted in each case, which, after settling and decantation of the alcohol, were dissolved again in a little water and precipitated by pouring into strong alcohol. Much coloring matter and sugar was held in solution, as proved by evaporation of the alcoholic mother-liquors. The precipitates were then thoroughly dehydrated with absolute alcohol, washed with ether, and dried at 110°. The spring-wheat extract thus yielded 6.9289 grams of substance, the winter wheat 8.7517 grams. As these preparations were unquestionably very impure

and no practicable method of purification existed which could be carried out without great loss of substance, the nitrogen in each was determined and the protein calculated by multiplying the result obtained by 6.25. The spring-wheat preparation was thus found to contain 3.07 per cent of nitrogen, equal to 19.19 per cent of protein, and the winter-wheat preparation 5.15 per cent nitrogen, equivalent to 32.18 per cent. The amount of proteose and peptone thus found in the extract from the spring wheat was 1.3297 grams, which equals 0.213 per cent of the flour, and in the winter-wheat extract 2.8063 grams, equal to 0.432 per cent of the flour.

The sodium chloride extract contained, therefore, the following amounts of protein:

	Spring wheat.	Winter wheat.
	P. ct.	*P. ct.*
Globulin	0.624	0.625
Albumin	0.391	0.359
Coagulum	0.269	0.223
Proteose	0.213	0.432
	1.497	1.639

The remainder of the protein matter of the seed forms the gluten. The proportion of gliadin and glutenin in this gluten was determined in the following manner:

200 grams of spring-wheat flour made from the entire seed and a like quantity of a similar winter-wheat flour were each made into a dough and thoroughly washed with water as long as starch was removed. The gluten thus obtained, after freeing from loosely adhering moisture, was weighed and exactly one-half dried at 110° to constant weight. The spring wheat was thus found to yield 12.685 per cent dry gluten, the winter-wheat flour 11.858 per cent.

The other half of the gluten was cut up very fine and extracted as thoroughly as possible with alcohol of 0.90 sp. gr. The alcoholic extract was then evaporated to small volume, cooled, and the solution decanted from the precipitated protein. This precipitate was then extracted with ether and dried at 110°. From the spring-wheat gluten 4.3379 per cent and from the winter wheat 4.2454 per cent of alcohol-soluble protein were obtained.

The residue extracted with alcohol was then dried at 110° and weighed. The spring-wheat gluten contained 7.80 per cent insoluble in alcohol, the winter wheat 7.504 per cent, reckoned on the wheat. Nitrogen was then determined in these residues dried at 110°, as well as in the dried gluten

and also in the original flours. The washings of the glutens were collected in jars and allowed to settle, the sediments washed with water and with very strong alcohol and dried and weighed. The nitrogen in each was then determined. The results of these determinations and deductions drawn from them are given in table 20.

TABLE 20.—*Amount of the various proteins of the wheat kernel.*

	Spring wheat.	Winter wheat.
	P. ct.	*P. ct.*
Total nitrogen in the flour	1.950	1.940
Total gluten in the flour	12.685	11.858
Part of gluten insoluble in alcohol	7.800	7.504
Per cent of nitrogen in gluten	12.010	12.000
Total nitrogen in gluten in per cent of flour	1.5222	1.4230
Total nitrogen in residue of gluten insoluble in alcohol	0.8245	0.7346
Total nitrogen extracted by alcohol	0.6977	0.6884
Gliadin (N × 5.68), assuming 17.60 per cent nitrogen in gliadin	3.9630	3.9100
Gliadin by direct weighing	4.3379	4.2454
Nitrogen in sediment from washing gluten	0.2239	0.1552

	Spring wheat.		Winter wheat.	
	Nitrogen.	Protein.	Nitrogen.	Protein.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*
Glutenin	0.8245 × 5.68	4.683	0.7346 × 5.68	4.173
Gliadin	0.6077 × 5.68	3.963	0.6884 × 5.68	3.910
Globulin	0.1248	0.624	0.1148	0.625
Albumin	0.0657	0.391	0.0603	0.359
Coagulum	0.0453	0.269	0.0379	0.223
Proteose	0.0341	0.213	0.0791	0.432
From H_2O washings of gluten	0.2239 × 5.68	1.272	0.1552 × 5.68	0.881
	2.0050	11.415	1.8703	10 603

THE FORMATION OF GLUTEN.

Wheat, so far as known, is the only plant whose seeds contain protein matter separable in a coherent form from the other constituents by washing with water. When ground fine and mixed with a suitable quantity of water, it yields a dough from which a light, porous bread can be made. The importance of this fact in bread-making is so great that considerable attention has been paid to gluten by the chemists who have studied wheat proteins.

Reference has already been made (p. 45) to the statements of Weyl & Bischoff that the protein matter of the wheat kernel is *chiefly* a globulin substance, which in contact with water undergoes a change, presumably through

the influence of a ferment by which gluten results. If the statements of these investigators are examined no evidence will be found to support their view. What their reasons were for concluding that "myosin" formed nearly all the protein of the wheat kernel does not appear. In view of the results obtained by the writer, this statement is certainly erroneous. Direct treatment of the meal with alcohol yielded extracts containing gliadin in exactly the same amount as obtained from the gluten made from an equal quantity of flour, and extraction of either flour or gluten with alcohol, after complete exhaustion with sodium-chloride solution, also gave the same proportion of gliadin. This substance must therefore have existed in the seed, and, as it forms one-half of the gluten, it leaves the other half only as possibly derived from a globulin body through the influence of a ferment. If Weyl & Bischoff's view were correct, treatment of the flour with 10 per cent salt solution ought to alter the character and quantity of the gluten obtained, if not altogether to prevent its formation. This is not so, for the usual amount of gluten can readily be obtained from flour made into dough with 10 per cent sodium-chloride solution and then washed with the same until starch is removed.

Weyl & Bischoff next state that "with the aid of a 15 per cent rock-salt solution the flour was extracted until no protein could be detected in the extract; the residue of the meal kneaded with water then gave no gluten. *If the globulin substance is extracted, no formation of gluten takes place.*" It has been found that this is true if the flour is stirred up with a large quantity of salt solution, extracted repeatedly with fresh quantities of the same solution until no more protein is dissolved, and the excess of solution removed by allowing the residue to drain on a filter as completely as possible. If, however, wheat flour is mixed at first with just sufficient salt solution to make a firm dough, this dough may be washed indefinitely with salt solution, and will yield gluten as well and as much as if washed with water alone. This difference is due to the fact that when large quantities of salt solution are applied at once the flour fails to unite to a coherent mass and can not afterward be brought together, as is possible when treated with smaller quantities of solution.

Weyl & Bischoff then compare the formation of gluten to that of blood-fibrin from fibrinogen under the influence of a ferment.

Sidney Martin next advanced a somewhat similar theory of the formation of gluten from the proteins contained in the seed. He states that alcohol extracts from gluten but one protein substance; that this is soluble in hot water, but not in cold, and he therefore calls it an insoluble phytalbumose.

The residue of the gluten not dissolved by alcohol is uncoagulated protein, if the alcohol has not been allowed to act too long. This substance he

names gluten-fibrin. Martin further says that gluten dissolves almost completely in 0.2 per cent hydrochloric acid or 0.2 per cent potassium-hydroxide solution, leaving a small residue of fat. The solution gives a copious precipitate when neutralized, but the supernatant liquid still contains a quantity of protein which is the dissolved insoluble albumose. The whole of the gluten-fibrin is reprecipitated by neutralization—that is, it is wholly converted into an albuminate.

Martin states that by extracting flour with 76 to 80 per cent alcohol only fat is removed. This statement is certainly erroneous, for the writer has never failed in many experiments thus to extract this substance (gliadin) from the flour, and that, too, in the same amount and of the same properties and composition as from the gluten.

Martin concludes that insoluble albumose is not present as such in the flour. He then says:

> Before proceeding to mention its precursor, it will be well to state that 10 per cent sodium-chloride solution extracts from flour a large quantity of globulin and of albumose. This globulin is of the myosin type, coagulating between 55° and 60° C., and precipitated by saturation with sodium chloride and ammonium sulphate. Both the globulin and albumose are present in a much smaller quantity in the watery extract of the flour.

From this it is evident that Martin has fallen into the same error as Weyl & Bischoff, mistaking the albumin for a myosin-like globulin, and being greatly misled as to its amount. Continuing, Martin says:

> The direction of the evidence is to show that the insoluble albumose is formed from the soluble. Moreover, I think that the globulin is transformed into the gluten-fibrin, for I have been able to obtain from the globulin in solution a body having the same reactions as the gluten-fibrin.

What this evidence is which by its direction shows that the insoluble albumose is derived from the soluble is not clear, and Martin makes no further statements on this point. That a body should be obtained from the solution containing the globulin which had the same reactions as the "gluten-fibrin" is not surprising, for the insoluble products derived from nearly all globulins have no characteristic reactions, being merely soluble in dilute acids and alkalis and precipitated by neutralization in the same way as "gluten-fibrin." Martin then states his theory of the formation of gluten in the following scheme:

$$\text{Gluten} = \begin{cases} \text{Gluten-fibrin—precursor, globulin.} \\ \text{Insoluble albumose—precursor, soluble albumose.} \end{cases}$$

This can not be a correct representation of the formation of gluten, for it has been shown to be founded on two erroneous observations—first, that alcohol does not extract protein matter from the flour when applied directly, and, second, that at least one-half the protein matter of the seed is a myosin-like globulin.

The results obtained by the author and described in this paper have led to the conclusion that no ferment-action is involved in the formation of gluten; that but two protein substances are contained in the gluten, glutenin and gliadin, and that these exist in the wheat kernel in the same form as in the gluten, except that in the latter they are combined with water in an amount equal to about twice the weight of the dried protein. The reasons for this opinion are, first, that alcohol extracts the same protein and in the same amount, whether applied directly to the flour, to the gluten, or to flour previously extracted with 10 per cent sodium-chloride solution; second, that 0.2 per cent potassium-hydroxide solution extracts glutenin of uniform composition and properties from flour which has been extracted with alcohol or with 10 per cent sodium-chloride brine and then with alcohol as it extracts from gluten which has been exhausted with alcohol.

Both glutenin and gliadin are necessary for the formation of gluten, as may be seen from the following experiments: A portion of flour was washed completely free from gliadin by means of alcohol of 0.90 sp. gr., next with stronger alcohol, finally with absolute alcohol, and air-dried. The residue was then rubbed up fine until all lumps were removed, and water carefully added and a dough made of the mass. A tolerably coherent dough was thus obtained, but much less elastic and tough than that produced from the untreated flour. This dough was then washed with water on a sieve, using every precaution to obtain a gluten, but none was formed.

In another experiment 7.5 grams of very finely ground air-dried gliadin were mixed intimately with 70 grams of fine corn-starch and distilled water added. A plastic dough was thus produced, but it had no toughness. On adding a little 10 per cent sodium-chloride solution, the dough became at once tough and elastic. This was then washed with great care on a sieve with cold water, a little 10 per cent sodium-chloride solution being added from time to time, but in spite of every precaution no gluten was obtained.

The following experiment shows that the gliadin used was capable of forming gluten when glutenin was present, and also that salts have a marked influence on the toughness of the resulting dough. Two portions of flour weighing 100 grams each were taken, and after adding 5 grams of gliadin to one both were made into dough with the same quantity of water. The two doughs presented marked differences. That to which gliadin had been added was much tougher and more yellow than the other. They were then washed with water as long as starch separated. The gluten was dried superficially by wiping with a cloth and weighed in the moist state. That from 100 grams of flour to which 5 grams of gliadin had been added weighed 44.55 grams; that from 100 grams of flour alone weighed 27.65 grams. The moist glutens were dried at 110° to constant weight, and both yielded

the same proportion of dry gluten, viz, 34.6 per cent. The yield of dry gluten was accordingly in the first case 15.41 grams and in the second 9.56 grams. The difference, 5.85 grams, shows that the added gliadin was fully recovered in the gluten.

The figures show that these proteins combine with about twice their weight of water in forming gluten. The fact that the added gliadin entered so readily and completely into the formation of gluten indicates that it exists in the seed as such and undergoes no chemical change during extraction and drying.

The properties observed in testing the separated gliadin show how it acts in forming gluten and explain many of the points observed by others and attributed to a ferment-action.

When treated with distilled water in small amount, the fine-ground air-dry gliadin at once forms a sticky mass, which, on adding more distilled water, dissolves to a turbid solution. If, however, a very little sodium chloride is added to distilled water and this applied to gliadin that has been first moistened with pure water, a very coherent, viscid mass results, which adheres to everything it touches and can be drawn out into long threads. If the gliadin is moistened with 10 per cent sodium-chloride solution and then treated with a larger quantity of this solution, the substance unites to a plastic mass, which can be drawn out into sheets and strings, but is not adhesive. From this it is evident why Ritthausen, in washing flours which gave a fluid gluten, obtainable only in small quantity and with great difficulty, found that the addition of calcium sulphate to the wash-water rendered the gluten much more coherent and easily obtainable. The gliadin is thus proved to be the binding material which causes the particles of flour to adhere to one another, thus forming a dough. But the gliadin alone is not sufficient to form gluten, for it yields a soft and fluid mass, which breaks up entirely on washing with water. The insoluble glutenin is probably essential by affording a nucleus to which the gliadin adheres and from which it is not mechanically carried away by the wash-water.

The behavior of the gliadin toward 10 per cent sodium-chloride solution shows why no gluten was obtained by Weyl & Bischoff from flour extracted with this solvent. The gliadin had under these conditions no adhesive qualities, and therefore was unable to bind the flour into a coherent mass. If, however, the salt solution is added in small quantities and the flour kneaded and pressed, the particles are brought together and then adhere tenaciously.

SUMMARY.

The proteins of the wheat kernel are *gliadin*, insoluble in neutral aqueous solutions, but distinguished from all the others by its ready solubility in neutral 70 per cent alcohol; *glutenin*,[1] a protein having a similar elementary percentage composition to gliadin, soluble in very dilute acid and alkaline solutions, but insoluble in dilute alcohol or neutral aqueous solutions and yielding a wholly different proportion of decomposition products when boiled with strong acids; *leucosin*, an albumin-like protein, freely soluble in pure water and coagulated by heating its solution to 50° to 60°; a globulin similar in composition and properties to many globulins found in other seeds, and one or more proteoses which are present in very small quantity. It has also been shown that the proteins obtained from the embryo of the wheat are the globulin, albumin, and proteose above mentioned, and that these form nearly all of the protein substance of this part of the seed. It thus appears that these three proteins are contained chiefly in the embryo, and that gliadin and glutenin form nearly the whole of the proteins of the endosperm, or over 80 per cent of the total protein matter of the seed.

It is possible that a part of the albumin, globulin, and perhaps minute quantities of the proteose are contained also in the endosperm, for these proteins are always found in flour from which, in the milling process, the embryo is very nearly completely separated. The uncertainty, however, as to the completeness of this separation makes it questionable whether or not the small amount of these proteins found in the best flour is not due to the presence of more or less of the embryo that escaped separation in the milling process.

The flour of wheat differs from that of other seeds in forming a dough when moistened with sufficient water, which, when washed with more water, loses its starch, and finally yields a tough elastic mass, long known as wheat gluten. This gluten contains the greater part of the protein matter of the seed, together with a little starch, fat, lecithin, and phytocholesterin, and possibly some carbohydrate substance or substances of as yet unknown character. These non-protein substances are probably not united with one another in the gluten, but are mechanically mixed. The quantity of starch that remains in the gluten depends on the thoroughness of the washing, while the other substances owe their presence largely to their insolubility in water. The chief constituents of the gluten are the two proteins, gliadin and glutenin, the relative proportions of which vary with the variety of wheat from

[1] This is the protein which Ritthausen called "gluten-casein."

which the flour is made. The character of the gluten and the commercial value of the flour depend to a large extent on the proportion of gliadin to glutenin.

In the moist gluten these proteins are present combined with about twice their weight of water, which is gradually lost on exposure to dry air or at an elevated temperature.

The gliadin and glutenin are present as such in the seed and are not, as was formerly supposed, derived from other protein substances through the action of an enzyme. This is shown by the fact that they may be obtained directly from the flour by the same treatment as that which yields them from the gluten and under conditions which preclude the action of an enzyme.

GLIADIN.

Gliadin is the most important of the five proteins above mentioned, not only on account of its influence on the character of the gluten, and therefore on the quality of the flour for domestic purposes, but also on account of its unusual physical properties and chemical constitution.

Gliadin, unlike most other protein substances, is freely soluble in relatively strong ethyl alcohol. Although gliadin is wholly insoluble in absolute alcohol and but slightly soluble in water, it dissolves in *dilute* alcohol, the solubility increasing with increasing concentration of alcohol until a certain strength is reached, when the solubility diminishes until, by absolute alcohol, it is no longer dissolved. Exactly what strength of alcohol dissolves the largest proportion of gliadin has never been determined, but the maximum solubility is attained with about 70 per cent of alcohol by volume.

Gliadin is also soluble in other alcohols, as methyl, propyl, and benzyl alcohols, in phenol and paracresol, and also in glacial acetic acid.

Gliadin is somewhat soluble in pure water, but less so in water containing salts, though not wholly insoluble in solutions containing 10 per cent of sodium chloride. Very dilute acid or alkaline solutions dissolve gliadin to solutions which, by neutralizing, yield the gliadin apparently unchanged. By stronger solutions of acids or alkalis the gliadin is altered, as is the case with all other native proteins.

All the usual color tests given by other protein substances are obtained with gliadin, which therefore contains the groups that give rise to these reactions. Gliadin is much less easily converted into insoluble products than are most other proteins. Its solution in 70 per cent alcohol can be boiled for an indefinite time, and even concentrated until much of the alcohol has been removed, without forming insoluble products. On heating with very weak alcohol or with water this protein is gradually altered and becomes insoluble in stronger alcohol, but the coagulated gliadin thus formed is in

appearance unlike the heat coagulum formed by most other proteins. Proteins of similar behavior to gliadin are found in the seeds of other cereals, such as rye, barley, maize, oats, and sorghum. That found in rye is probably identical with the gliadin of wheat, for a rigid comparison has not yet revealed any difference. Both have the same ultimate composition, the same solubility, the same physical properties, and yield the same amount of ammonia and glutaminic acid on hydrolysis. The identity of the two, however, is not certain, and with our present knowledge can not be established. The proteins of the other cereals above mentioned are distinctly different, though similar, proteins.

Proteins characterized by such ready solubility in strong alcoholic solutions as are those found in these cereals have not been obtained from the seeds of any other plants.

The ultimate composition of gliadin has been fixed within narrow limits by the accordant analyses of several investigators as follows :

	Günsberg.	Ritthausen.	Osborne & Voorhees.	Nasmith.	König & Rintelen.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.67	52.76	52.72	52.39	52.70
Hydrogen	6.83	7.10	6.86	6.84	7.62
Nitrogen	17.62	18.01	17.66	17.47	17.77
Sulphur	.....	0.85	1.03	1.12	0.95
Oxygen	22.88	21.28	21.73	22.18	20.96
	100.00	100.00	100.00	100.00	100 00

Gliadin yields on hydrolysis a larger amount of ammonia, glutaminic acid, and proline than any other protein yet examined, no glycocoll or lysine, and a relatively small amount of histidine and arginine, as the following analysis shows :

Products of hydrolysis of gliadin.

	P. ct.		*P. ct.*
Glycocoll	0.00	Tyrosine	1.20
Alanine	2.00	Cystine	0.45
Amino-valerianic acid	0.21	Lysine	0.00
Leucine	5.61	Histidine	0.61
Proline	7.06	Arginine	3.16
Phenylalanine	2.35	Ammonia	5.11
Aspartic acid	0.58	Tryptophane	present
Glutaminic acid	37.33		
Serine	0.13	Total	65.81

Although gliadin gives a strong Molisch reaction, which is commonly considered to indicate the presence of a carbohydrate group, it gives no furfurol when distilled with hydrochloric acid, as does ovalbumin, which has been

proved to contain such a group. Other evidence than that given by the Molisch reaction is required before the presence of a carbohydrate group in this protein can be assumed.

Nearly two-thirds of the total sulphur in gliadin is split off as sulphide by boiling with alkalis, which would indicate the possibility that all the sulphur is contained in a cystine complex. The amount of cystine isolated is far below that required by such an assumption. The determination, however, of this substance is not quantitative, and the figure given does not necessarily prove that all of the sulphur may not be contained in a cystine-yielding complex.

The specific rotation of gliadin in 80 per cent by volume ethyl alcohol, according to determinations by the writer, which agree closely with those of others, is $(\alpha)\frac{20^\circ}{D} = -92.3^\circ$.

The specific rotation in solvents of alcoholic nature has been recently determined by Mathewson, who gives the following data:

	$(\alpha)\frac{40^\circ}{D}$		$(\alpha)\frac{40^\circ}{D}$
Methyl alcohol, 70 per cent.	— 95.65°	Phenol, 70 per cent	— 123.15°
Ethyl alcohol, 70 per cent.	— 91.95°	Phenol, anhydrous	— 131.77°
Ethyl alcohol, 60 per cent.	— 96.66°	Paracresol	— 121.00°
Ethyl alcohol, 50 per cent.	— 98.45°	Benzyl alcohol	— 53.10°
Propyl alcohol, 60 per cent.	— 101.10°	Glacial acetic acid	— 78.60°

The amount of gliadin in different varieties of wheat differs to a large extent, as shown by a considerable number of determinations that have been recorded. Thus Teller found in a sample of Canadian white winter wheat 2.74 per cent, in one of Oregon white winter wheat 2.85 per cent, and in a red spring wheat from South Dakota 8.15 per cent. In other varieties of wheat he and others have found quantities falling between these figures.

Although the total quantity of gliadin in the sample of red wheat above mentioned was nearly three times as great as in that of the white wheat, its percentage of the total proteins was much more uniform, being 42.5 and 34 per cent respectively. This appears to be generally true for most of the wheats that have been examined.

Thus Shepard has found in 13 samples of durum wheat that the gliadin formed from 40.2 to 48.6 per cent of the total protein ($N \times 5.7$), in 9 samples of Northwestern spring wheat from 44.2 to 49 per cent, in 4 samples of Kansas hard winter wheat from 40 to 50.7 per cent, and in 3 samples of soft winter wheat from 40 to 47.4 per cent. The figures given by others are similar, though a few fall between somewhat wider limits. It would seem safe to say that in the majority of wheats the gliadin forms from 40 to 50 per cent of the total proteins, and that its absolute amount depends chiefly on the total protein content of the seed.

GLUTENIN.

This protein was first described by Taddei under the name of zymom. Liebig, as well as Dumas & Cahours, named it "plant-fibrin;" Ritthausen called it "gluten-casein;" Weyl & Bischoff considered it to be an albuminate form of a myosin-like globulin. On the ground of priority and the fact that the relations to animal proteins, which gave rise to the various names subsequently applied to this body, have been proved to have no foundation, it would be desirable to return to Taddei's original name and in future call this protein zymom. Unfortunately this name is derived from the Greek word ζύμη, a ferment, and as the results of this and all other subsequent investigations of this subject show that the supposed ferment-changes do not occur in the formation of gluten, it seems appropriate to call it glutenin, a name suggested by S. W. Johnson. Glutenin is next in importance to gliadin, for in most varieties of flour it is present in nearly equal amount. It is so nearly insoluble in water and alcohol that it is a question whether the slight traces that are dissolved by these solvents from carefully purified preparations are not due to traces of gliadin, which it is very difficult to separate from it. In hot dilute alcohol glutenin is slightly soluble and separates from such solutions on cooling. When freshly precipitated and in the hydrated condition, it is very readily dissolved by extremely dilute acids or alkalis and is precipitated from such solutions on neutralization. Glutenin can be separated from the other constituents of the seed only by solution in dilute alkalis or acids, and it is necessary to filter the solutions, from which it is finally precipitated, perfectly clear in order to separate the associated non-protein substances. This filtration is accomplished with great difficulty unless it is preceded by thorough extraction of the crude glutenin with alcohol and ether, whereby fats and lecithins are removed.

Glutenin contains all the groups which give rise to the usual color reactions of the proteins. No protein similar to glutenin in physical and chemical properties has yet been found in any other seed. Although its ultimate composition is nearly the same as gliadin, the proportion of the various products of hydrolysis which it yields is very different, as may be seen from the following analyses:

The ultimate composition of glutenin.

	P. ct.
Carbon	52.34
Hydrogen	6.83
Nitrogen	17.49
Sulphur	1.08
Oxygen	22.26
	100.00

The following products of hydrolysis have been obtained by boiling glutenin with strong hydrochloric acid:

Products of hydrolysis of glutenin.

	P. ct.		P. ct.
Glycocoll	0.89	Tyrosine	4.25
Alanine	4.65	Cystine	0.02
Amino-valerianic acid	0.24	Lysine	1.92
Leucine	5.95	Histidine	1.76
α-proline	4.23	Arginine	4.72
Phenylalanine	1.97	Ammonia	4.01
Aspartic acid	0.91	Tryptophane	present
Glutaminic acid	23.42		
Serine	0.74		59.66

Glutenin is qualitatively distinguished from gliadin by yielding both glycocoll and lysine, and quantitatively by yielding less proline, glutaminic acid, and ammonia and more alanine, tyrosine, and arginine. In other respects the differences are not great.

The amount of glutenin varies greatly in different samples of wheats, but usually forms about 40 per cent of the total protein of the seed. Teller has found in a sample of Canadian white winter wheat 3.64 per cent of glutenin and in an Oregon white winter wheat 3.82 per cent, while in a red spring wheat from South Dakota he found 8.04 per cent. Although the total amount of glutenin in these wheats was very different, the relative amounts were nearly the same, being 45.2 and 46.5 per cent of the total proteins in the white winter wheats and 42 per cent in the red spring wheat. Other winter wheats differed but little from other spring wheats both in the total and relative amount of glutenin which they contained. In most of the wheats that he examined the glutein formed from 40 to 45 per cent of the total proteins. Shepard, who analyzed a much larger number of samples, found a wider variation in the relative proportion of glutenin, from 34.2 to 46.8 per cent of the total proteins, and a narrower range in its total amount, the latter falling between 3.41 and 7.10 per cent.

LEUCOSIN.

Leucosin forms about 0.3 to 0.4 per cent of the wheat kernel. Although the entire seed contains but little leucosin, the embryo contains a relatively large proportion, since about 10 per cent of the commercial "germ meal" consists of this protein. As this "germ meal" contains more or less of the endosperm and outer coats of the seed, the embryo contains somewhat more than 10 per cent. Leucosin is an albumin, for it is soluble in pure water and coagulated by heating its solution. It has been mistaken by several observers for a myosin-like globulin, owing to the fact that its temperature of coagulation falls near to that of such globulins found in animal tissues,

and also to the fact that its solutions are precipitated by saturating them with sodium chloride or with magnesium sulphate. That it is soluble in pure water and not held in solution by small quantities of salts is shown by the fact that a solution containing a considerable quantity of leucosin when subjected to prolonged dialysis and then evaporated to dryness, and the leucosin burned off at a low temperature left a residue of mineral matters weighing less than a milligram. Leucosin is much more readily precipitated by ammonium sulphate than are the albumins of animal origin, for by adding an equal volume of a saturated solution of this salt to solutions containing leucosin the latter is almost completely precipitated.

Leucosin resembles the animal proteins in ultimate composition, in the proportion of its products of hydrolytic decomposition, and in its physical properties more closely than it resembles most of the seed proteins yet studied. It seems not improbable that this is because it is a constituent of the tissues of the embryo, for its physiological functions are unquestionably different from those of the stored-up food proteins of the endosperm.

Proteins having the same ultimate composition and, so far as known, the same properties as leucosin are found in the seeds of other cereals. Whether these are identical or not can not be determined by any means now available.

The ultimate composition of leucosin is shown by the following figures, which are the average of accordant analyses of several preparations:

Composition of leucosin.

	P. ct.
Carbon	53.02
Hydrogen	6.84
Nitrogen	16.80
Sulphur	1.28
Oxygen	22.06
	100.00

Leucosin yields the following amounts of the several products of hydrolysis when boiled with strong hydrochloric acid:

Products of hydrolysis of leucosin.

	P. ct.		P. ct.
Glycocoll	0.94	Tyrosine	3.34
Alanine	4.45	Lysine	2.75
Amino-valerianic acid	0.18	Histidine	2.83
Leucine	11.34	Arginine	5.94
α-proline	3.18	Ammonia	1.41
Phenylalanine	3.83	Tryptophane	present
Aspartic acid	3.35		
Glutaminic acid	6.73		50.32

Leucosin probably contains a relatively large proportion of tryptophane, for in a series of comparative tests made with a large number of different

proteins the intensity of its glyoxylic acid reaction was greater than that of any of the other proteins.

Leucosin also gave the strongest reaction with the Molisch test of any of a large number of different proteins examined under like conditions, but whether or not it contains a carbohydrate group must be shown by other evidence.

THE GLOBULIN.

The seeds of wheat contain about 0.6 per cent of a protein which is insoluble in water, but soluble in neutral saline solutions. This globulin is chiefly contained in the embryo from which 5 per cent was extracted by sodium-chloride solution. The preparations of the globulin that were made from the embryo contained nucleic acid, while those from the whole seed contained none. These nucleic acid compounds from the embryo behaved like nucleates, for their proportion of nucleic acid was not constant, but varied with the conditions of preparation. It seemed most probable that the nucleic acid combines with the basic protein to form salts in the same manner as other acids are known to do, and that such combinations with the globulin still retained the solubility characteristic of the globulin. It is quite possible that other insoluble compounds containing more nucleic acid may have existed in the embryo or have been formed during extraction, so that the amount of globulin obtained may not have equaled that actually present in the embryo. The fact that the globulin from the whole seed was free from nucleic acid, while that from the embryo was not, was probably due to the presence of a much larger proportion of protein insoluble in salt solution in the whole seed compared with that in the embryo, so that the nucleic acid united with the insoluble protein instead of with the globulin, as happened when the embryo was extracted.

This globulin is very similar to, if not identical with, that found in the seeds of rye and barley. It contains over 18 per cent of nitrogen, and resembles, in composition and properties, many of the globulins found in large proportion in many other seeds.

Owing to the difficulty encountered in preparing large quantities of this globulin, the products of its hydrolysis have not been studied.

Its ultimate composition is shown by the following figures, which are the average of several analyses of preparations from the whole seed:

	P. ct.
Carbon	51.03
Hydrogen	6.85
Nitrogen	18.39
Sulphur	0.69
Oxygen	23.04
	100.00

This globulin is precipitated by saturating its solutions with magnesium sulphate, but not with sodium chloride. Dissolved in 10 per cent sodium-chloride solution, it is partly coagulated by boiling, but is not coagulated at temperatures below 100°.

THE PROTEOSE.

The wheat kernel yields a very small amount of proteose when extracted with water. Whether this is an actual constituent of the seed or is formed from the other proteins during its extraction and isolation was not definitely ascertained. This proteose, like leucosin and globulin, is chiefly found in the embryo. No preparations were made from the entire seed in sufficient quantity to permit of analysis or examination of its properties.

The proteose obtained from the embryo appeared to be a mixture of two or more substances. A part was precipitated by saturating its solution with sodium chloride; a part was not.

The analyses of these two parts showed the low percentage of carbon characteristic of proteoses obtained by peptic digestion. Analyses of preparations thus obtained gave the following results:

	Proteose insoluble in saturated NaCl solution.	Proteose soluble in saturated NaCl solution.
	P. ct.	*P. ct.*
Carbon.....	49.94	48.99
Hydrogen.....	6.80	6.85
Nitrogen.....	17.08	16.89
Sulphur	1.24	1.10
Oxygen.....	24.94	26.17
	100.00	100.00

THE GLUTEN.

The proteins of the wheat kernel differ from those that have been found in any other seed by the fact that they may be largely separated as a coherent elastic mass by washing the dough made from the flour under a gentle stream of water. This protein mass has long been known as gluten. It consists chiefly of gliadin and glutenin in combination with about twice their weight of water, together with more or less starch, which can not be wholly removed by washing, and also some fat, cholesterin, and lecithin. The non-protein constituents usually form about 20 to 25 per cent of the crude gluten. The protein constituents of the gluten, except for the fact that they are combined with water, are present in the same form in the gluten as in the grain.

Their proportion is also nearly the same, for practically the same amount of gliadin can be obtained from the gluten as from the flour, if allowance is made for the small amount of gluten that is mechanically carried away in the process of washing out the starch. This loss is largely made up by the presence of non-protein constituents in the gluten, so that the weight of the dried gluten usually corresponds closely with the amount calculated for the sum of gliadin and glutenin, as computed from the nitrogen belonging to these proteins.

The gluten is not formed from globulins by the action of a ferment in a manner analogous to the formation of fibrin from fibrinogen, as was asserted at one time. The observations on which this supposition was founded were incorrect.

The glutenin probably forms the nucleus to which the gliadin adheres, and thus binds the gluten proteins into a coherent elastic mass.

Both gliadin and glutenin are necessary for the formation of gluten, for a dough made with starch and gliadin, or one made with flour from which the gliadin has been extracted with alcohol, yields no gluten when washed with water. That the gliadin is capable of taking part in gluten formation under such conditions is shown by the fact that when dry and finely ground gliadin was added to wheat flour the amount of gluten obtained on subsequently washing out the starch was increased by the full amount of the gliadin that was added.

THE NUTRITIVE VALUE OF THE WHEAT PROTEINS.

It has recently been shown that in the process of digestion the protein molecule is very largely broken down into amino-acids, and that the animal forms from these, by the processes of assimilation, the proteins of its blood and tissues. How this change is effected is not known, nor is it known whether the food protein is converted into the body protein and then oxidized and eliminated, or is partly converted into body protein and partly burned directly in the form of amino-acids. It is also a question whether or not the animal has the power to convert one amino-acid into another, and thus obtain material suitable for the construction of its own body protein.

Directly connected with these important problems are the facts presented by the determination of the relative amounts of the different amino-acids yielded by the proteins of wheat flour, for these are used in enormous quantities as food by man, and, as an examination of the analyses of their decomposition products show, present marked differences from similar analyses of all the other food proteins thus far examined.

As gliadin and glutenin together form about 85 per cent of the proteins of wheat flour, they deserve especial consideration in this respect. If it is

assumed that these two proteins are present in equal quantities in wheat flour, an assumption that in most cases is nearly correct, the relative proportion of the amino-acids yielded by the proteins of wheat flour is closely shown by the following figures, which are the mean of those found for gliadin and glutenin. In comparison with these are given the figures, at present available, for some of the other forms of food protein. Such a comparison is at present unsatisfactory, owing to the lack of data now available, but it will serve a useful purpose in indicating the wide differences between these food proteins, and will emphasize the importance of obtaining more information in regard to this question.

Products of hydrolysis of some food proteins.

	Wheat gluten.	Milk casein.	Egg albumin.	Zein, maize.	Excelsin, Brazil nut.	Phaseolin, white bean.	Beef muscle.	Halibut muscle.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Glycocoll	0.44	0.00	0.00	.....	0.60	0.55	.....	.....
Alanine	3.33	0.90	2.10	.....	2.33	1.80	.....	.....
Amino-valerianic acid.	0.23	1.00	.....	.....	1.51	1.04	.....	.....
Leucine	5.78	10.50	6.10	.....	8.70	9.56	.....	.....
Proline	5.65	3.10	2.25	.....	3.65	2.77	.....	.....
Phenylalanine	2.16	3.20	4.40	.....	3.55	3.25	.....	.....
Aspartic acid	0.75	1.20	1.50	.....	3.85	5.24	.....	.....
Glutaminic acid	30.38	11.00	9.10	16.87	12.94	14.54	11.1	8.9
Serine	0.44	0.23	.....	.....	.....	0.38	.....	.....
Tyrosine	2.73	4.50	1.10	.....	3.16	2.17	.....	.....
Cystine	0.24	0.06	0.20	.....	.....	.....	.....	.....
Lysine	0.96	5.80	.....	0.00	1.64	3.59	.....	.....
Histidine	1.19	2.59	.....	0.81	1.47	1.97	.....	.....
Arginine	3.94	4.84	.....	1.82	16.02	4.72	.....	.....
Ammonia	4.56	1.95	1.63	3.61	1.80	2.06	.....	.....
Tryptophane	present	1.50	present	0.00	present	present	.....	.....

Owing to the lack of data, it is not yet possible to compare the relative proportion of the amino-acids which the food proteins yield except in respect to glutaminic acid, ammonia, arginine, histidine, and lysine. The amount of glutaminic acid which the gluten proteins yield is far greater than that yielded by any of the other food proteins, with the exception of gliadin from rye and hordein from barley. The proteins of the legumes and nuts which are used as food yield from 15 to 20 per cent of glutaminic acid, so that the mean amount of this amino-acid from the wheat protein is nearly twice as large. The same also is true of ammonia.

The proportion of arginine from wheat gluten is relatively small compared with that from most other seed proteins, many of which yield from 10 to 16 per cent of this base.

The proportion of lysine is likewise small, especially compared with that obtained from the leguminous seeds. The amount of histidine, however, does not differ very greatly from that of the other seed proteins. What significance these differences have in respect to the nutritive value of these different proteins must be determined by future investigation, for it has only very recently been discovered that such differences exist.

That a molecule of gliadin can have the same nutritive value as one of casein would seem impossible if one molecule of food protein is transformed into one of tissue protein, for in the former lysine is wholly lacking, and glutaminic acid, ammonia, and proline are in great excess over the amount required to form any of the tissue proteins of which we know. It would seem probable that either the animal requires a variety of food, so that the relative proportion in which the amino-acids are available for its use shall correspond more nearly to its requirements, or that only a small part of these amino-acids are converted into its tissue proteins and the rest oxidized as such. It is possible that feeding experiments with proteins of known character in respect to the relative proportions of their decomposition products will throw light on these important questions.